UNIT 7 LIPID METABOLISM

Structure

7.1 INTRODUCTION

Lipids are a heterogeneous group of organic compounds. The major dietary lipids for humans are animal and plant triacylglycerols, sterols and membrane phospholipids. You may recall reading about their structure and properties earlier in Unit 2. Here, in this unit, we shall focus on their metabolism. The process of lipid metabolism involves synthesis and degradation of the lipid stores. It also involves the production of the structural and functional lipids characteristic of individual tissues. This unit gives an overview of lipid metabolism at the cellular level, which would provide enough background for understanding the aberrations with regard to lipid metabolism and its related diseases.

The first part of the unit, section 7.2 – Lipid Metabolism I – focuses on fatty acid metabolism i.e. issues/reactions related to degradation and the synthesis of fatty acids – saturated, unsaturated – in our body. The second part of the unit, section 7.3 – Lipid Metabolism II – looks at the metabolism of neutral fats, phospholipids, cholesterol etc. In addition, you will also find information regarding hyperlipoproteinemias, ketosis. What do these terms mean? Read and find out in this unit.

Objectives

After studying this unit, you will be able to:

- explain how fatty acids are oxidized for the production of energy,
- describe the synthesis of fatty acids,
- discuss the metabolism of triacylglycerols, phospholipids and cholesterol,
- relate the cholesterol and lipoprotein metabolism to hyperlipidemia, and
- discuss the significance of eicosanoids in human nutrition.

7.2 LIPID METABOLISM – I

You learnt earlier in Unit 5 that the lipids are absorbed through the intestine. As these molecules are oils, solubilization (emulsification) of dietary lipids is accomplished via bile salts that are synthesized in the liver and secreted from the gall bladder.

The emulsified fats are then degraded by pancreatic lipases. These enzymes, secreted into the intestine from the pancreas, generate free fatty acids and a mixture of mono- and diacylglycerols from dietary triacylglycerols.

Following absorption of the products of pancreatic lipase by the intestinal mucosal cells, the resynthesis of triacylglycerols occurs. The triacylglycerols are then solubilized in lipoprotein complexes (complexes of lipid and protein) called *chylomicrons*. Triacylglycerols synthesized in the liver are packaged into VLDLs and released into the blood directly. Chylomicrons from the intestine are then released into the blood via the lymphatic system for delivery to the various tissues for storage or production of energy through oxidation. The triacylglycerol components of VLDLs and chylomicrons are hydrolyzed to free fatty acids and glycerol in the capillaries of adipose tissue and skeletal muscle.

Finally, what is the fate of fatty acid? Let us find out.

7.2.1 Oxidation of Fatty Acids

Fatty acids released from chylomicrons and VLDL are transferred across cell membranes by passive diffusion, which depends upon the concentration gradient. Their oxidation consists of: (a) activation, (b) transport into the mitochondrial matrix, and (c) reactions of β-oxidation. Let us next learn about these three stages.

a) *Activation of fatty acids*

Fatty acids cannot undergo oxidation in the form in which they are normally present in the body. They must be converted into a form which can be catabolized. This process is called *activation* and fatty acid is converted into an active intermediate. Fatty acids must be activated in the cytoplasm before being oxidized in the mitochondria. This activation is catalyzed by *fatty acyl CoA synthetase*. At least three acyl CoA synthetases, each specific for a particular size (length) of fatty acid are known. Let us see what are these.

1) *Acetyl CoA synthetase:* This acts on acetate and low molecular weight fatty acids,
2) *Medium chain acyl CoA synthetase:* This acts on fatty acids with 4-11 carbon atoms, and
3) *Acyl CoA synthetase:* This acts on fatty acids with 6 to 20 carbon atoms.

The activity of acetyl CoA synthetase in muscles is restricted to the mitochondrial matrix. The medium-chain acyl CoA synthetase occurs only in liver mitochondria, where medium chain fatty acids (obtained from digestion of dietary triacylglycerols and transported by the portal blood) are metabolized. Acyl CoA synthetase, the major activating enzyme, occurs on the outer mitochondrial membrane surface and in endoplasmic reticulum.

Activation of fatty acids results in the formation of an ester between the carboxyl (COOH) group of the fatty acid and SH group of the coenzyme A, forming fatty acid ester of coenzyme A. The overall reaction of activation is:

$$\underset{\text{(fatty acid)}}{RCOO^- + ATP + CoASH} \rightleftharpoons \underset{\text{(fatty acyl CoA)}}{R - \overset{\overset{O}{\|}}{C} - CO\ SCoA + AMP + PP}$$

The reaction favours the formation of fatty acyl CoA, as the pyrophosphate formed is hydrolyzed by the enzyme *pyrophosphatase*: $PP_i + H_2O \longrightarrow 2P_i$

Thus, activation of a fatty acid molecule requires expenditure of two high-energy phosphate bonds.

b) *Transport of acyl CoA to mitochondrial matrtix*

You learnt above that the activation of fatty acids takes place largely in the cell cytoplasm. However, the enzyme required for oxidation is present in the mitochondria. The activated long chain fatty acids, therefore, must be transported into the mitochondria. However, the mitochondrial membrane is impermeable (not allowing passage) to fatty acyl CoA ester. Hence, the transport of acyl CoA is achieved by *carnitine* (γ-β-hydroxy-γ-trimethylammonium butyrate). Two *carnitine acyl transferases* are involved in acyl CoA transport: *carnitine palmitoyl transferase I (CPT I)*, located on the outer surface of the inner mitochondrial membrane and *carnitine palmitoyl transferase II (CPT II)* located on the inner surface.

The activated long chain fatty acyl CoA ester reacts with carnitine in the presence of *carnitine palmitoyl transferase I*. CoA is released and the fatty acid forms complex with carnitine called acyl-carnitine. This is able to penetrate the inner mitochondrial membrane and gain access to β-oxidation systems of enzymes. Here, another enzyme *carnitine-acylcarnitine translocase* acts as an inner membrane exchange transporter. Thus, acylcarnitine is transported in, coupled with the transport out of one molecule of carnitine, as shown in Figure 7.1. This carnitine is generated when acylcarnitine reacts with CoA to form once again fatty acyl CoA, catalyzed by *carnitine palmitoyl transferase II,* located on the inside of the mitochondrial membrane. Once inside the mitochondrion, the fatty acyl-CoA is a substrate for the β-oxidation machinery, as discussed in the next step. The role of carnitine in transport of fatty acids into the mitochondrial matrix is given below in Figure 7.1.

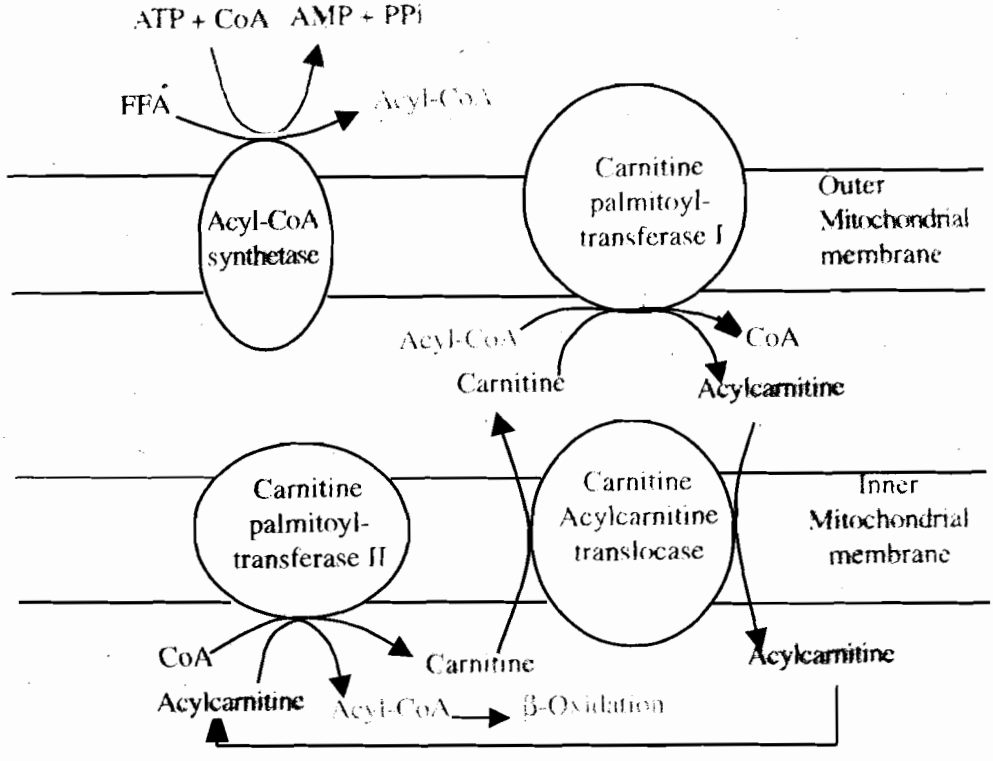


Figure 7.1 : Carnitine cycle

c) *β-oxidation*

The major pathway for fatty acid oxidation is *β-oxidation.* The process of fatty acid oxidation is termed as β-oxidation since it occurs through the sequential removal of 2-carbon units (as acetyl CoA) by oxidation at the β-carbon position (between α(2) and β(3) carbon atoms) of the fatty acyl-CoA molecule. The reactions take place entirely in mitochondrial matrix.

Oxidation of a saturated acyl CoA with an even number of C atom to acetyl CoA requires a repeated, sequential action of the following four enzymes and the steps are highlighted herewith and in Figure 7.2:

i) Acyl CoA dehydrogenase

ii) Enoyl CoA hydratase

iii) β-hydroxy acyl CoA dehydrogenase

iv) Acetyl CoA acyltransferase (β-ketothiolase or thiolase).

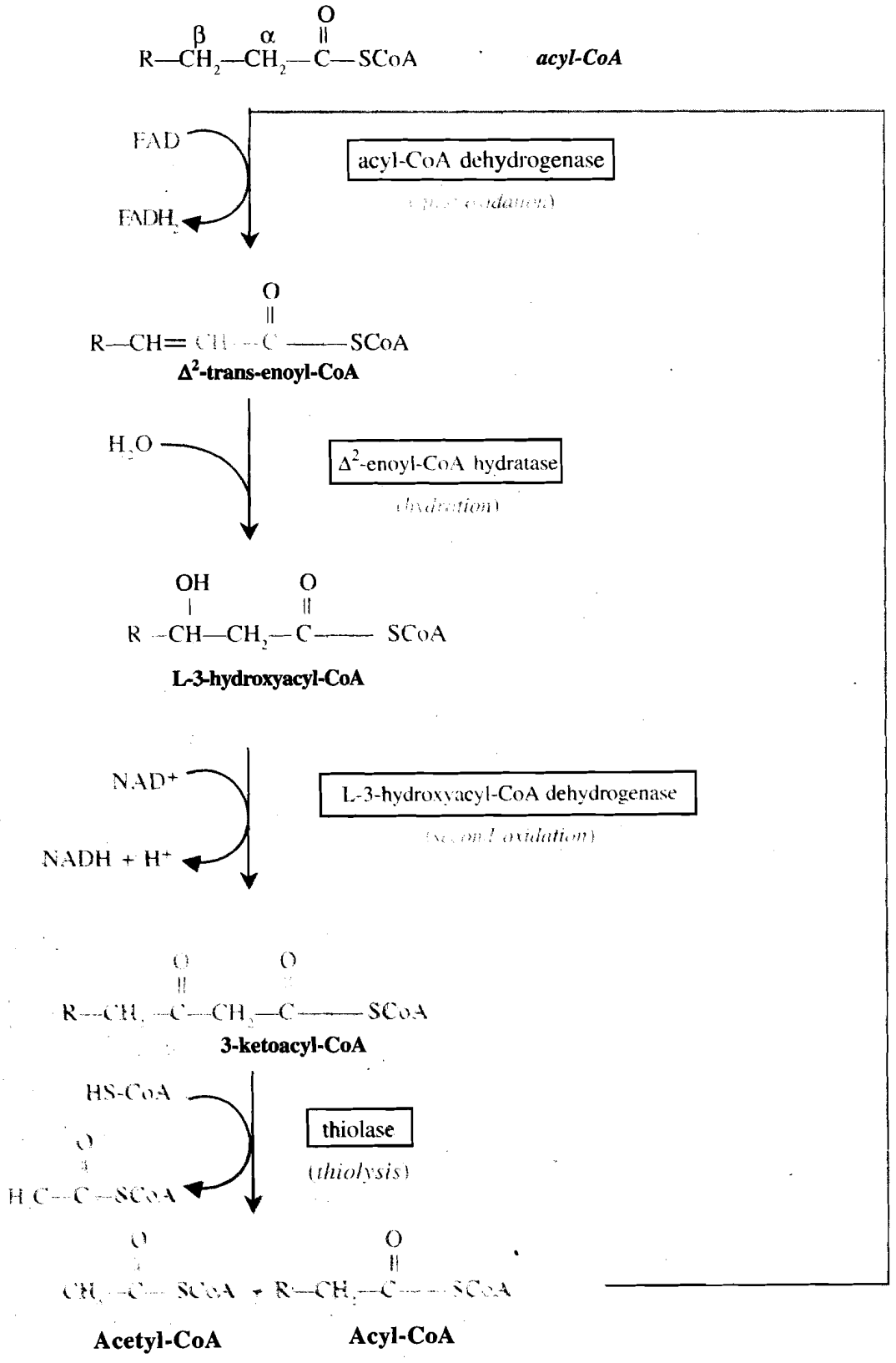


Figure 7.2 : β-oxidation of fatty acids

i) Acyl CoA dehydrogenase dehydrogenates acyl CoA (i.e. removes two H^+) at the α and β C atoms. This causes unsaturation to give α, β unsaturated acyl CoA (or Δ^2 unsaturated acyl CoA). The dehydrogenases are flavoproteins and contain a tightly bound molecule of flavin adenine dinucleotide (FAD as the coenzyme). The electrons of the $FADH_2$ via another flavoproteins are transferred to the respiratory chain to give 2 ATP molecules. The Δ^2 double bond formed has a *trans* geometrical configuration. The double bonds naturally occurring in fatty acids are in *cis* form.

ii) Enoyl-CoA hydratase hydrates the Δ^2 unsaturated acyl CoA. This enzyme has broad specificity and can act on α, β (or Δ^2) unsaturated CoA in *trans* or *cis* configuration. The product formed is L (+)β-hydroxyacyl CoA. When the trans double bond is hydrated, the D-isomer is formed with *cis* double bond.

iii) β-hydroxyacyl CoA dehydrogenase oxidizes β-hydroxyacyl CoA by an NAD^+ - linked reaction that is absolutely specific for L-stereoisomer. The electrons from the NADH generated are passed on to NADH dehydrogenase of the respiratory chain and finally 3 ATP molecules are formed.

iv) Acetyl CoA acyl transferase (or thiolase) catalyzes a thiolytic cleavage (i.e. involving SH group) and gives acetyl CoA and acyl CoA, which is shortened by 2 C atoms.

The entire sequence of the oxidation of fatty acid, right from the activation stage to β-oxidation is given in Figure 7.3. The shortened fatty acyl CoA from one cycle is further oxidized in successive passes until it is entirely converted to acetyl CoA.

It is important to note that a majority of natural lipids contain an even number of carbon atoms. A small proportion that contain odd numbers, upon complete β-oxidation, yield acetyl-CoA units plus a single mole of propionyl-CoA. The propionyl-CoA is converted, in an ATP-dependent pathway, to succinyl-CoA. The succinyl-CoA can then enter the citric acid cycle for further oxidation.

Before we conclude, let us look at the energetics of the β-oxidation process, discussed above. Table 7.1 presents the outcome. As is evident, 2 mole equivalents of ATP are used during the activation of the fatty acid. On the other hand, the electrons of the $FADH_2$ are transferred to the respiratory chain to give 2 ATP molecules. The electrons from the NADH generated are passed on to the respiratory chain and finally 3 ATP molecules are formed. Oxidation of acetyl CoA gives 12 moles of ATP (each acetyl CoA in citric acid cycle gives 3 NADH and 1 $FADH_2$ and 1 GTP for total 12 ATP).

Table 7.1 : Energetics of β-oxidation

Reaction	Moles of ATP gained / lost
Activation reaction	−2
First dehydrogenation (FAD)	+2
Second dehydrogenation (NAD)	+3
Oxidation of acetyl CoA	12

Let us understand this by taking an example of palmitic acid (C16). Palmitoyl CoA will give 8 acetyl CoA molecules (one acetyl CoA gives 12 ATP, hence 8 molecules will give 96 ATP) and will undergo β oxidation 7 times (since 2 acetyl CoA molecules are formed in the last cycle). Activation step is only once. The overall ATP yield therefore is:

Activation step	..	-2×1	=	-2
β-oxidation	..	7×5	=	35
Acetyl CoA	..	8×12	=	96
				129

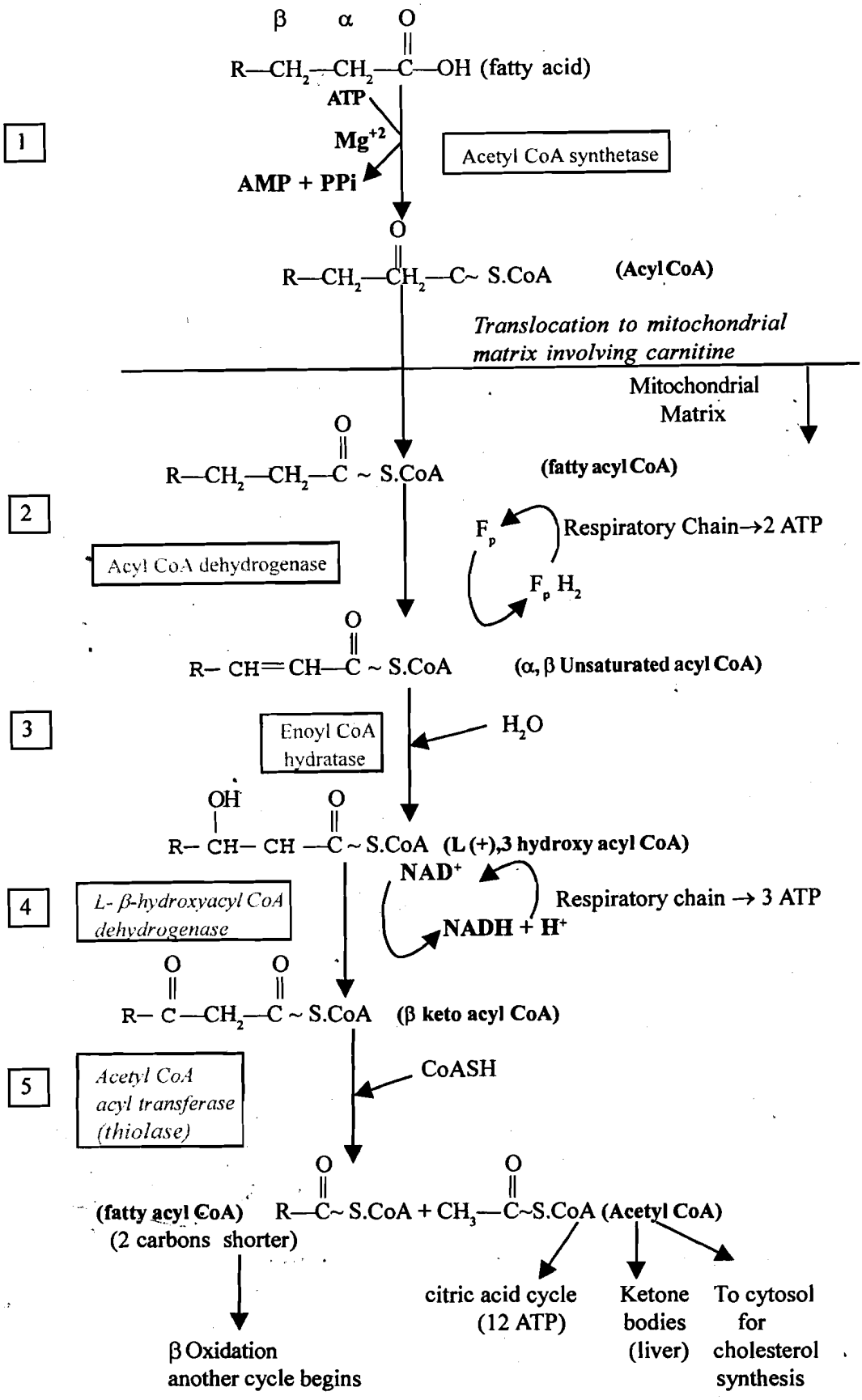


Figure 7.3 : Fatty acid activation, transport and β-oxidation

Complete oxidation of one molecule of palmitic acid gives 129 ATP molecules. Similarly, the net result of the oxidation of one mole of oleic acid (an 18-carbon fatty acid) will be 146 moles of ATP (2 mole equivalents are used during the activation of the fatty acid).

With our discussion above, we have come to the end of our study on fatty acid oxidation. Next, we shall learn about the oxidation of unsaturated fatty acid.

7.2.2 Oxidation of Mono and Poly Unsaturated Fatty Acids

The oxidation of unsaturated fatty acids is essentially the same process as for saturated fats, as discussed above, except when a double bond is encountered. In such a case, the bond is isomerized by a specific *enoyl-CoA isomerase* and oxidation continues. In the case of linoleate (linoleic acid), the presence of the C-12 unsaturation results in the formation of a dienoyl-CoA during oxidation. This molecule is the substrate for an additional oxidizing enzyme, the NADPH requiring *2,4-dienoyl-CoA reductase*.

Thus, oxidation of unsaturated fatty acids require Δ^3 *cis* or *trans* Δ^2 *trans enoyl CoA isomerase* and NADPH dependent *2,4 dienoyl CoA reductase*, in addition to the enzymes of β-oxidation. Hence, these two enzymes are also referred to as *additional* or *auxillary enzymes*.

To help you understand the process and the role of the auxiliary enzymes in the oxidation process of unsaturated fatty acids, we have included the oxidation of oleic acid and linoleic acids– two of the unsaturated fatty acids here in Box 1. We have presented these oxidation reactions only to help your understanding on the subject and not primarily for examination purposes. This is extra reading material. Do not get bogged down by these reactions. In fact you should practice these reaction using linolenic acid (C-18; Δ 9,12,15) and arachidonic acid (C-20, Δ 5,8,11,14) also.

Box 1 : Oxidation of Unsaturated Fatty Acids

Oxidation of unsaurated fatty acids given in Figures 7.4 and 7.5 are involved herein. In Figure 7.4, you can see that oleic acid (C18) has a double bond between carbon 9 and 10. All naturally-occurring double bonds are in *cis* configuration. Usual 3 turns of β-oxidation results in the formation of 3 molecules of acetyl CoA and oleic acid becomes a C_{12} fatty acid, with the original *cis* double bond now being between carbons 3 and 4, i.e. it is Δ^3 *cis* enoyl CoA. This is an inactive substrate in β-oxidation. So another enzyme Δ^3 *cis* (or *trans*)→ Δ^2 *trans enoyl CoA isomerase* converts the *cis* double bonds to *trans* (which is required in β-oxidation) and puts the double bond in positions 2-3. Now Δ^2 *trans* enoyl CoA is formed and it goes through five more turns of β-oxidation forming 6 acetyl CoA molecules.

Next, let us look at the oxidation of linoleic acid. As evident in Figure 7.5, linoleic acid has 18 carbon atoms, with 2 double bonds in positions 9-10 and 12-13, respectively. It goes through three turns of β-oxidation forming 3 acetyl CoA molecules and a C_{12} fatty acid with 2 *cis* double bond in 3-4 and 6-7 positions. This is acted upon by the auxillary enzyme Δ^3 *cis (or trans)* Δ^2 *trans enoyl CoA isomerase* forming Δ^2 *trans-* Δ^6*-cis* dienoyl CoA. Now one more turn of β-oxidation takes place, removing one molecule of acetyl CoA and forming Δ^6*-cis enoyl CoA*. This is acted upon by *acyl CoA dehydrogenase* and an extra trans double bond is introduced in 2-3 position forming Δ^2 *trans-*Δ^4*-cis* dienoyl CoA. Now a second auxillary enzyme Δ^2 *trans-*Δ^4*cis dienoyl CoA reductase,* utilizing NADPH, reduces one double bond and forms Δ^3*-trans* enoyl CoA, while the configuration (trans) of the double bond is suitable for β-oxidation position 3-4 is not suitable. So the first auxillary enzyme, Δ^3 *cis (or trans)*→ Δ^2 *trans enoyl CoA isomerase* changes the position and forms Δ^2 *trans* enoyl CoA. This goes through four more turns of β-oxidation forming 5 more acetyl CoA molecules. Thus with the help of these two auxillary enzymes, linoleic acid can be oxidized completely to 9 molecules of acetyl CoA. In this way, all unsaturated fatty acids can undergo β-oxidation.

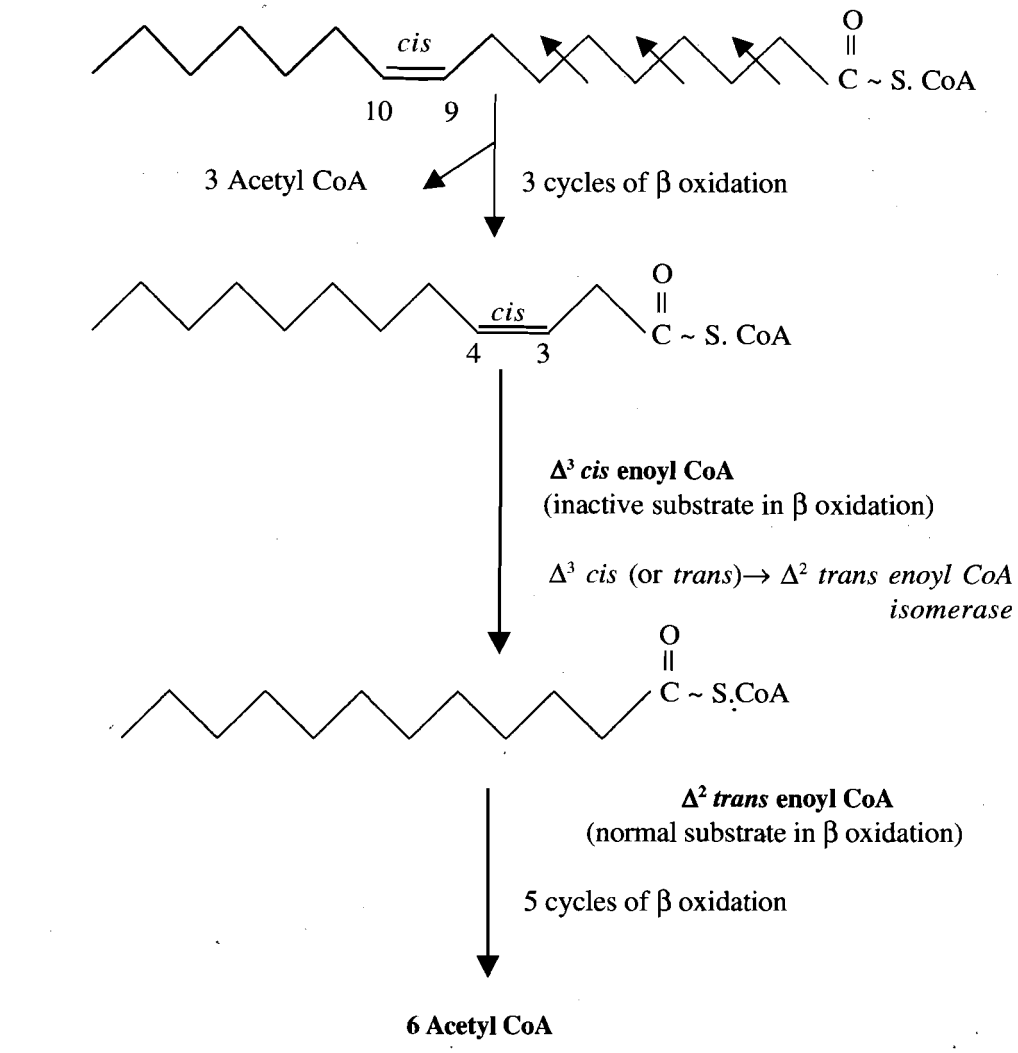
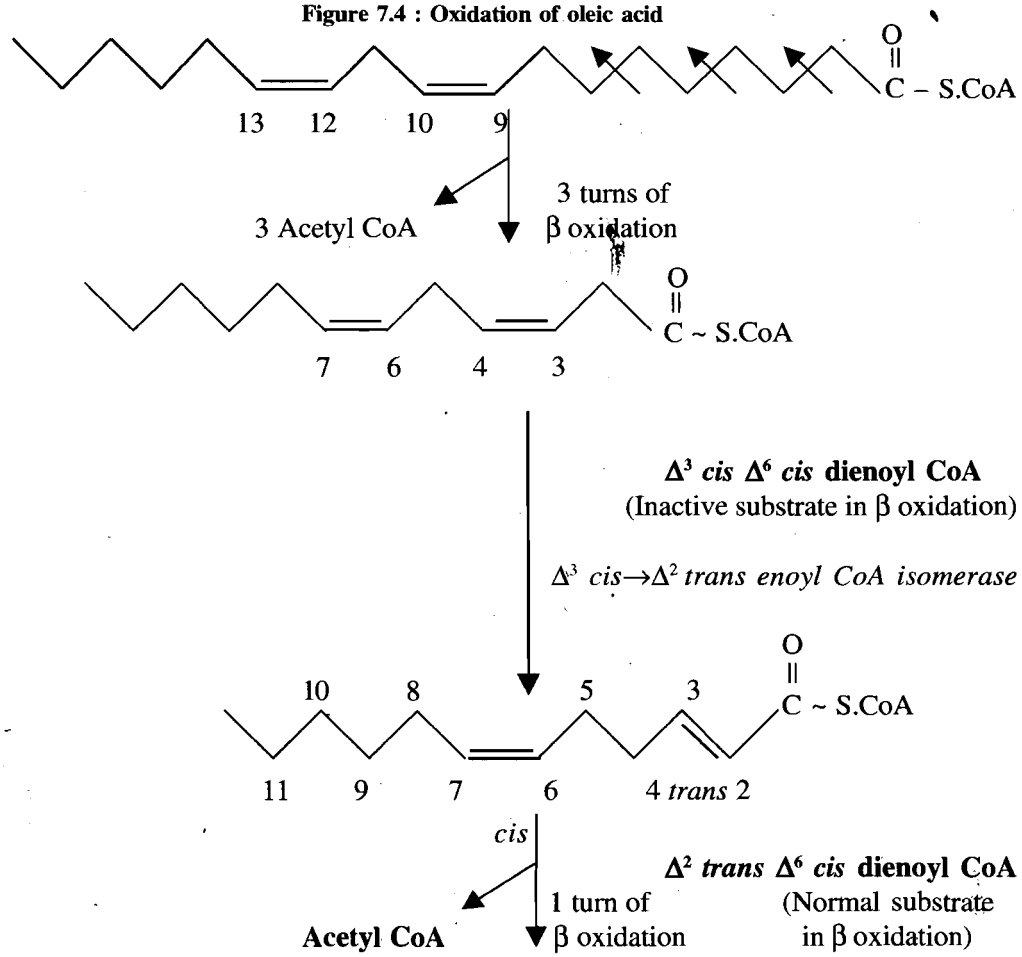


Figure 7.4 : Oxidation of oleic acid



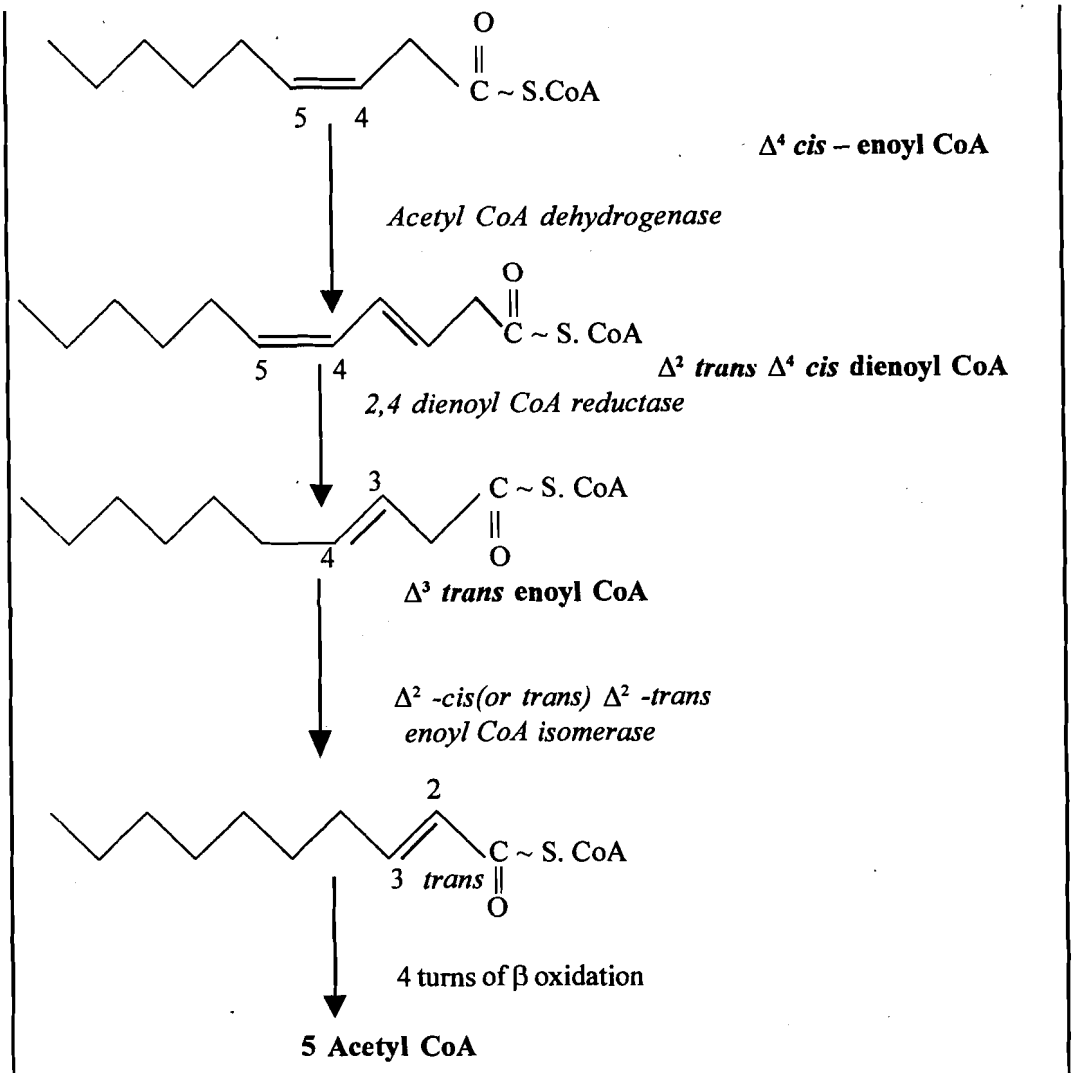


Figure 7.5 : Oxidation of linoleic acid

So we have seen that the oxidation of fatty acids yields acetyl CoA, which is a major source of useful metabolic energy. Now, what is the metabolic fate of acetyl CoA? Read and find out in the next section. But, first let us recapitulate what we have learnt so far.

Check Your Progress Exercise 1

1) List the three steps involved in the oxidation of fatty acids? Give the enzymes involved in the process of activation of fatty acids.

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2) Name the enzymes involved in the transfer of fatty acyl CoA ester? Briefly discuss the role of carnitine in transfer of fatty acids.

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3) What do you understand by the term β-oxidation? Highlight the role of 4 enzymes and steps involved.

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4) How many molecules of ATP are obtained from a β-oxidation of 1 molecule of stearic acid (C18)?

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5) How is the oxidation of poly unsaturated fatty acids (oleic, linoleic) different from oxidation of fatty acids?

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Having studied the oxidation of fatty acid, next we shall learn about the synthesis of fatty acids.

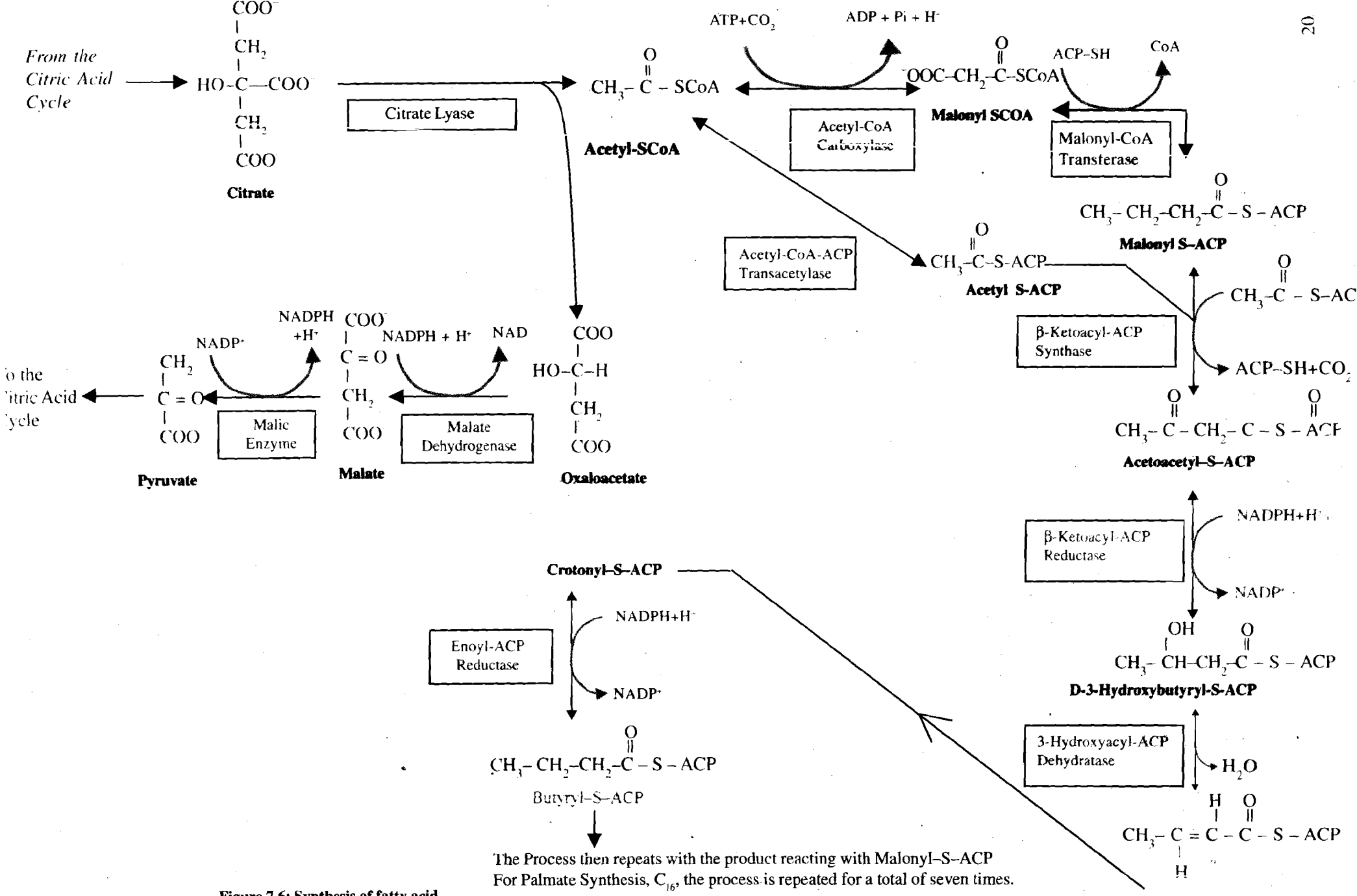
7.2.3 Lipogenesis – Synthesis of Fatty Acids

You would notice that acetyl CoA can react "reversibly" in the degradation or synthesis of lipids. Above, we saw that fatty acids are degraded to acetyl CoA. Interestingly, the formation of lipids too starts with acetyl CoA. Let us see how?

When there is an oversupply of dietary carbohydrates (CHOs), the excess CHOs are converted to triacylglycerols (you already know that triacylglycerols constitute molecules of glycerol to which three fatty acids have been esterified. In other words, fatty acids are stored primarily in the adipose tissue as triacylglycerol). Further individuals on low fat diets also convert glucose to triacylglycerol, which is stored. This involves the synthesis of fatty acids from acetyl CoA and the esterification of fatty acids in the production of triacylglycerol. The process is called *'lipogenesis'*. Infact, the sequence of reactions involved in the formation of lipids is known as *lipogenesis*. Lipogenesis is not simply the reversal of the fatty acid degradation, but does starts with acetyl CoA and does build up by the addition of two carbons units.

The fatty acid synthesis occurs in the cytoplasm in contrast to the degradation (oxidation), which you may recall reading earlier, occurs in the mitochondria. The major lipogenic tissues are the intestine, liver and adipose tissue. During lactation, the mammary gland also becomes a major site for lipogenesis and places a heavy demand on a continuing supply of glucose for the synthesis of milk lipids.

The metabolic reactions involved in the synthesis of fatty acid are illustrated step by step in Figure 7.6. The synthesis occurs in the cytosol from acetyl CoA. *This is also called de novo (afresh) synthesis.*



The Process then repeats with the product reacting with Malonyl–S–ACP
For Palmate Synthesis, C_{16}, the process is repeated for a total of seven times.

Figure 7.6: Synthesis of fatty acid

When glucose is abundant and the amount of citrate in the mitochondrial matrix exceeds the demand by the citric acid cycle, the excess citrate is transported out of the mitochondria into the cytosol by *tricarboxylate translocase*. Here, as you can see in Figure 7.6, the citrate is cleaved by *citrate lyase* to provide the acetyl group for fatty acid synthesis. Besides acetyl CoA, NADPH is also required. The NADPH necessary for fatty acid synthesis derives from the conversions of malic enzyme, *glucose-6-P dehydrogenase*, *gluconate-6-P dehydrogenase* and NADP-*dependent isocitrate dehydrogenase*.

The key regulating enzyme of lipogenesis is *acetyl-CoA carboxylase*. It catalyzes the synthesis of malonyl-CoA from acetyl-CoA and CO_2. In the formation of malonyl CoA via acetyl CoA carboxylase, biotin is tightly bound to the enzyme as a prosthetic group and acts as a carrier of a carboxyl group that is transferred to acetyl CoA. The formation of malonyl CoA signals the beginning of the synthesis of fatty acid.

The synthesis of fatty acids from acetyl-CoA and malonyl-CoA is carried out by *fatty acid synthase, FAS*. Actually, many of the enzymes for the fatty acid synthesis are organized into a multienzyme complex called *fatty acid synthase*. The sequence of reactions catalyzed by this enzyme, as presented in Figure 7.6, can be represented by the following seven reactions.

In the *first reaction*, acetyl CoA is added to a cysteine-SH group of the condensing enzyme (CE) domain:

$$\text{acetyl CoA + CE-cys-SH} \rightarrow \text{acetyl-cys-CE + CoASH}$$

Mechanistically, this is a two-step process, in which the group is first transferred to the ACP (acyl carrier peptide), and then to the cysteine-SH group of the condensing enzyme domain.

In the *second reaction*, malonyl CoA is added to the ACP sulfhydryl group:

$$\text{malonyl CoA + ACP-SH} \rightarrow \text{malonyl ACP + CoASH}$$

This -SH group is a part of a phosphopantethenic acid prosthetic group of the ACP.

In the *third reaction*, the acetyl group is transferred to the malonyl group with the release of carbon dioxide:

$$\text{malonyl ACP + acetyl-cys-CE} \rightarrow \text{beta-ketobutyryl-ACP + } CO_2$$

In the *fourth reaction*, the keto group is reduced to a hydroxyl group by the *beta-ketoacyl reductase* activity.

$$\text{beta-ketobutyryl - ACP + NADPH + } H^+ \rightarrow \text{beta-hydroxybutyryl-ACP + } NADP^+$$

In the *fifth reaction*, the beta-hydroxybutyryl-ACP is dehydrated to form a *trans*-monounsaturated fatty acyl group by the beta-hydroxyacyl dehydratase activity:

$$\text{beta-hydroxybutyryl- ACP} \rightarrow \text{2-butenoyl-ACP + } H_2O$$

In the *sixth reaction*, the double bond is reduced by NADPH, yielding a saturated fatty acyl group two carbons longer than the initial one (an acetyl group was converted to a butyryl group in this case):

$$\text{2-butenoyl-ACP + NADPH + } H^+ \rightarrow \text{butyryl-ACP + } NADP^+$$

The butyryl group is then transferred from the ACP sulfhydryl group to the CE sulfhydryl:

butyryl - ACP + CE-cys-SH → ACP-SH + butyryl-cys-CE

This is catalyzed by the same transferase activity as was used previously for the original acetyl group.

The butyryl group is now ready to condense with a new malonyl group (third reaction above) to repeat the process.

When the fatty acyl group becomes 16 carbons long, a thioesterase activity hydrolyses it, forming free palmitate:

palmitoyl - ACP + H_2O → palmitate + ACP-SH

Go through these reactions carefully. Initially, they might seem a bit tough, but if you follow the sequence as presented in Figure 7.6, you will understand the process of synthesis better.

So you notice, the primary fatty acid synthesized by FAS is palmitic acid. Once an acetyl group and a malonyl group are bound to the fatty acid synthase, seven rounds of enzymatic reactions proceed for the synthesis of palmitic acid, which is then released from the complex. The overall reaction is as follows:

Acetyl CoA + 7 Malonyl CoA + 14 NADPH + 14 H^+
→ Palmitic acid + 8 CoASH + 14 $NADP^+$ + 6H_2O + 7CO_2

Palmitate is then released from the enzyme and can then undergo separate elongation and/or unsaturation to yield other fatty acid molecules. We shall learn about this next.

So we have seen that in the opposite of fatty acid degradation, which is located within the mitochondria, de novo synthesis of fatty acids takes place within the cytosol. *The primary fatty acid synthesized by FAS is palmitic acid.* Palmitic acid can be converted to other unsaturated fatty acids. Let us see how.

Synthesis of other unsaturated fatty acids

Palmitic acid may be converted to stearic acid (18:O) by elongation of the carbon chain. Desaturation of stearic acid produces oleic acid (C_{18} : 1 Δ 9). The enzymes are located in the mitochondria and endoplasmic reticulum and can use fatty acid of varying chain lengths and degrees of unsaturated as substrates. Desaturation occurs in the ER membranes as well and in mammalian cells involves 4 broad specificity *fatty acyl-CoA desaturases* (non-heme iron containing enzymes). These enzymes introduce unsaturation at C4, C5, C6 or C9. Since these enzymes cannot introduce sites of unsaturation beyond C9 they cannot synthesize either linoleate ($18:2^{C9,12}$) or linolenate ($18:3^{C9,12,15}$). These fatty acids must be acquired from the diet and are, therefore, referred to as essential fatty acids. Linoleic is especially important in that it is required for the synthesis of arachidonic acid. As we shall encounter later, arachindonate is a precursor for the eicosanoids (the prostaglandins and thromboxanes). It is this role of fatty acids in eicosanoid synthesis that leads to poor growth, wound healing and dermatitis in persons on fat free diets. Also, linoleic acid is a constituent of epidermal cell sphingolipids that function as the water permeability barrier in the skin.

Before we end our study on fatty acid degradation and synthesis, let us recapitulate the salient features of the two processes. Table 7.2 gives the comparison of fatty acid synthesis and degradation. This will help you understand the two processes better.

Table 7.2 : Comparison of fatty acid synthesis and degradation

	Synthesis	Degradation
Greatest flux through pathway	After CHO rich meal	In starvation
Hormonal state favouring pathway	High insulin / glucagon ratio	Low insulin/glucagon ratio
Major tissue site	Primarily liver	Muscle, liver
Subcellular location	Primarily cytosol	Primarily mitochondria
Carriers of acyl/acetyl groups between mitochondria and cytosol	Citrate (Mitochondria to cytosol)	Carnitine (cytosol to mitochondria)
Oxidation/reduction cofactors	NADPH	NAD^+, FAD
Two C donor/product	Malonyl CoA : donor of one acetyl group	Acetyl CoA : Product
Activator	Citrate	
Inhibitor	Fatty acyl CoA (inhibits acetyl CoA carboxylase)	Malonyl CoA inhibits carnitine acyltransferase
Product of pathway	Palmitate	Acetyl CoA

Next, we shall focus on a family of compounds called eicosanoids. What are eicosanoids? What is their role in the body? You may recall reading about them in Unit 2 sub-section 2.3.5. We suggest you look up this section again now. So then what do you find? Yes, eicosanoids are derived from polyunsaturated fatty acids. We shall learn about their metabolism next.

7.2.4 Metabolism of Eicosanoids

You already know now that prostaglandins and the related compounds such as thromboxanes and leukotrienes (collectively known as eicosanoids) are extremely potent compounds that elicit a wide range of physiologic responses. These compounds have extremely short half life and are produced in very small amounts. They have been compared to hormones in terms of their actions, but they differ from the true hormones in that they are formed in almost all tissues rather than in specialized glands. They generally act locally rather than after transport in the blood to distant sites of action. Prostaglandins are metabolized to inactive products at their site of synthesis and are not stored to any appreciable extent. Let us see how these eicosanoids are synthesized in our body, starting with prostaglandins. Here, we are not going into the details of the reactions involved in the synthesis of these eicosanoids. As dietitians, it is only important for us to understand that eicosanoids are derived from essential fatty acids (EFAs).

A) Synthesis of Prostaglandins

The dietary precursor of the prostaglandins is the essential fatty acid linoleic acid. It is converted to its immediate precursor of the prostaglandins – 20 C, PUFA containing 3, 4 or 5 double bonds. Arachidonic acid is the precursor of the predominant classes of prostaglandins.

B) Synthesis of Leukotrienes

Arachidonic acid is converted to a variety of hydroperoxy acids by a separate pathway involving a family of *lipoxygenases*. For e.g. neutrophils contain 5-lipoxygenase, which converts arachidonic acid to 5-hydroxy 6, 8, 11, 14 eicosatetraenoic acid (5 HPETE). 5 HPETE is converted to a series of leukotrienes.

Check Your Progress Exercise 2

1) With reference to lipogenesis, answer the following:

   a) Definition of lipogenesis

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   b) Key regulating enzyme

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   c) Conversion of Malonyl CoA to β-hydroxybutyryl ACP

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2) Comment on the statement, 'fatty acid synthesis is simply a reversal of fatty acid degradation'.

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3) How is palmitate converted into oleic acid?

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4) What do you understand by the term eicosanoids? Where are these derived from? Discuss the synthesis of prostaglandins.

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7.3 LIPID METABOLISM – II

Earlier in section 7.2, we got to know about the fate of fatty acids in our body and also how they are synthesized. Now, in this section we shall focus on the metabolism of lipids such as triacylglycerol, cholesterol, phospholipids etc. You have already learnt about the structure and properties of these compounds in Unit 2, section 2.2 and 2.3. Do look up this unit once again, as it will help you understand their metabolism better. We start with the metabolism of triacylglycerol.

7.3.1 Metabolism of Triacylglycerols

The synthesis of triacylglycerol takes place in the endoplasmic reticulum. In liver and adipose tissue, fatty acids in the cytosol obtained from the diet or from de novo synthesis of palmitic acid become inserted into the endoplasmic reticulum (ER) membrane. Fatty acids, you learnt earlier, are stored for future use as triacylglycerols in all cells, but primarily in adipocytes of adipose tissue. *Triacylglycerols constitute molecules of glycerol to which three fatty acids have been esterified.* The fatty acids incorporated

into triacylglycerols are activated to acyl-CoAs through the action of *acyl-CoA synthetases*. This is the same kind of reaction, which you have seen earlier in activation of fatty acid, prior to oxidation. A membrane bound *acyl CoA transferase* then esterifies two molecules of acyl-CoA with glycerol 3-phosphate, to form *phosphatidic acid* i.e.1,2-diacylglycerol phosphate. Thus phosphatidic acid contains glycerol with a fatty acid each in carbon 1 and 2 and phosphate group in carbon 3. The phosphate is then removed by *phosphatidic acid phosphatase* to yield 1,2-diacylglycerol, the substrate for addition of the third fatty acid. *Phosphatidic acid phosphatase* releases phosphate and in the membrane, 1,2-diacylglycerol is esterified with a third molecule of fatty acid.

In the intestine, triacylglycerol synthesis also occurs in the ER membrane, but the starting material is 2-monoacylglycerol (which is glycerol esterified with a fatty acid at carbon 2) as can be seen in Figure 7.7.

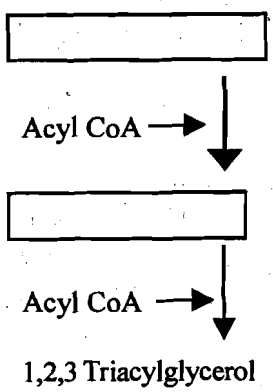
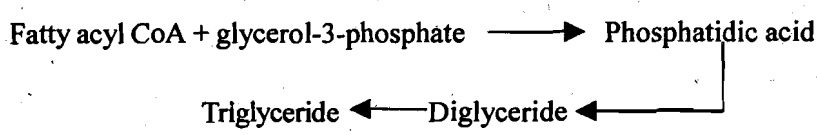


Figure 7.7 : Triacylglycerol synthesis

The summary of the reaction is as under:



In the liver and intestine, triacylglycerol is packaged into lipoproteins, which are then secreted into the circulation.

Next, let us learn about the synthesis of phospholipids.

7.3.2 Synthesis of Phospholipids

Phospholipids are synthesized by esterification of an alcohol to the phosphate of phosphatidic acid (1,2-diacylglycerol 3-phosphate). Most phospholipids have a saturated fatty acid on C-1 and an unsaturated fatty acid on C-2 of the glycerol backbone. The most commonly added alcohols (serine, ethanolamine and choline) also contain nitrogen that may be positively charged, whereas, glycerol and inositol do not. The major classifications of phospholipids are: *Phosphatidylcholine* (PC), *Phosphatidylserine* (PS), *Phosphatidylglycerol* (PG), *Phosphatidylethanolamine* (PE) and *Phosphatidylinositol* (PI).

Phosphatidylcholine, a major phospholipid constituent of membranes and lipoproteins is synthesized de novo in liver cells. The synthesis occurs in the ER and is linked, through 1,2-diacylglycerol, with the synthesis of triacylglycerol. Three compounds specifically involved in the synthesis of phosphatidyl choline are: a) choline, b) choline phosphate and c) cytidine diphosphatidyl choline (CDP-choline). The synthesis of phosphatidyl choline (with the pathway and the enzymes involved) is given in the Figure 7.8. As can be seen, choline is activated first by phosphorylation and then by coupling to CDP prior to attachment to phosphatidic acid. PC is also synthesized by the addition of choline to CDP-activated 1,2-diacylglycerol.

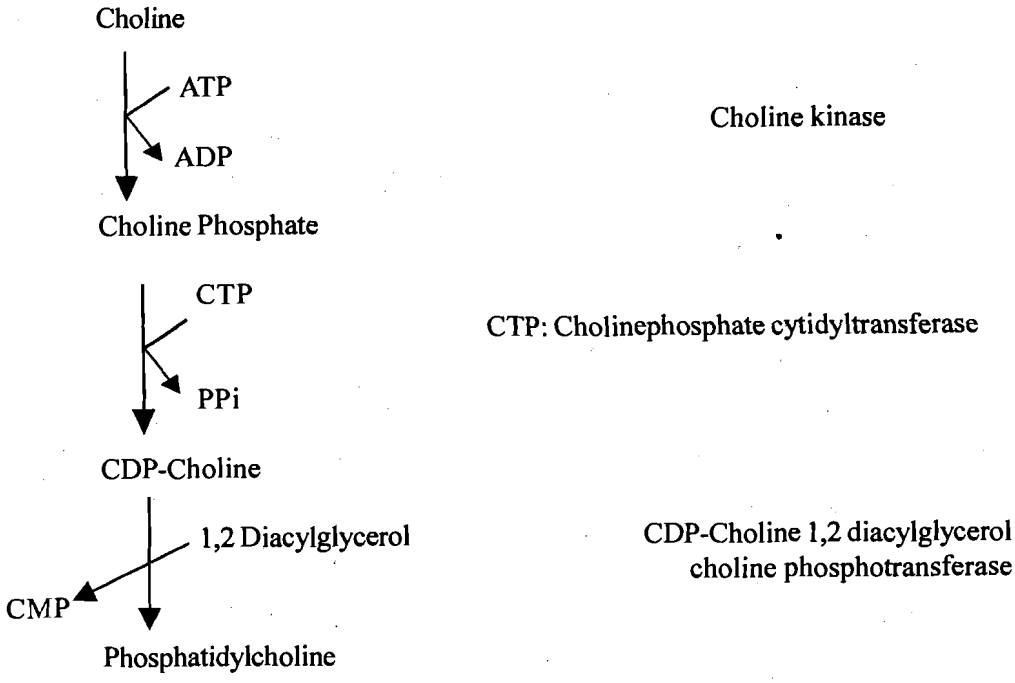


Figure 7.8 : Synthesis of phosphatidylcholine (PC)

A third pathway to PC synthesis, involves the conversion of either PS or PE to PC as shown in Figure 7.9. The conversion of PS to PC first requires decarboxylation of PS to yield PE, this then undergoes a series of three methylation reactions utilizing S-adenosylmethionine (SAM) as a methyl group donor.

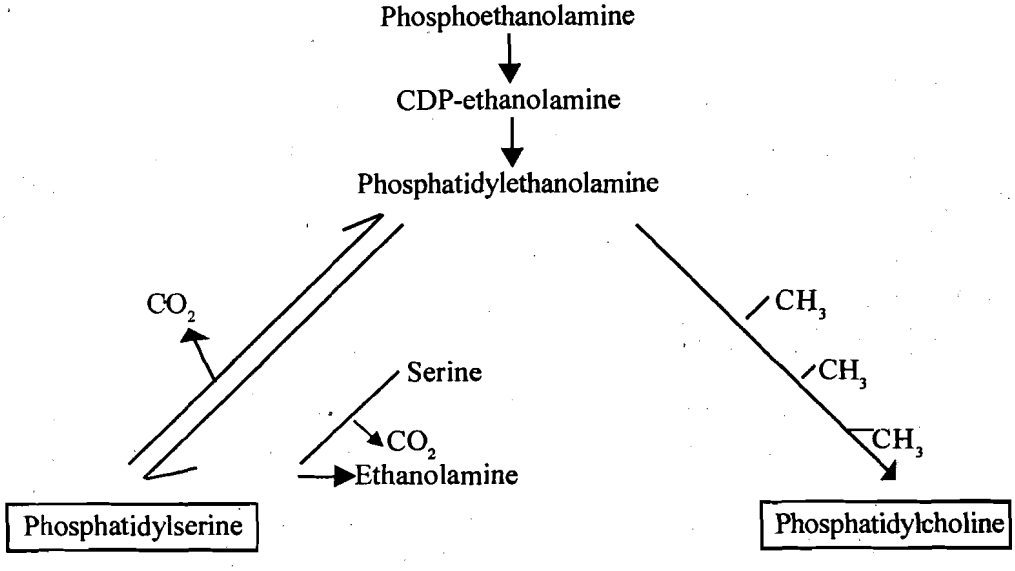


Figure 7.9 : Synthesis of phosphatidylserine (PS) and phosphatidylcholine (PC) from phosphoethanolamine (PE)

Phosphatidylserine arises by an exchange of the ethanolamine residue of phosphatidylethanolamine for a seryl group. Decarboxylation of the serine of phosphatidylserine reforms phosphatidylethanolamine. Three successive methylation reactions convert phosphatidylethanolamine to phosphatidylcholine. S-Adenosyl methionine is the methyl group donor.

We move on next to the metabolism of cholesterol.

7.3.3 Metabolism of Cholesterol

Cholesterol, as you may already know, is involved in two major biological processes a) It is a structural component of cell membranes, and b) Steroid hormones, vitamin D_3 (cholecalciferol) and the bile salts are derived from the parent compound.

Cholesterol is synthesized *de novo* in the liver and the intestinal epithelial cells and is also derived from dietary lipids. De novo synthesis of cholesterol is regulated by the amount of cholesterol and triglyceride in the dietary lipid. Let us learn how it is synthesized.

A) Cholesterol Biosynthesis in Liver and Intestinal Epithelium

The biosynthesis of cholesterol, a complex molecule with 27 carbon atoms, starts with the two-carbon atom compound acetyl-CoA which is converted to isopentenyl pyrophosphate (an isoprene derivative with five carbon atoms) and then squalene (30 carbon atoms), which is finally cyclized to cholesterol. It involves 32 different enzymes, some of which are soluble in the cytosol and others of which are bound to the ER.

The process has five major steps, which are listed herewith:

1) Acetyl-CoA is converted to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA)
2) HMG-CoA is converted to mevalonate
3) Mevalonate is converted to the isoprene based molecule, isopentenyl pyrophosphate (IPP), with the concomitant loss of CO_2
4) IPP is converted to squalene (through a series of steps)
5) Squalene is converted to cholesterol.

The pathway of cholesterol synthesis is presented in the Figure 7.10.

For those of you who would want to know a bit more of each of these steps as illustrated in Figure 7.10, the following description will be useful.

The acetyl-CoA utilized for cholesterol biosynthesis is derived from an oxidation reaction (eg, fatty acids or pyruvate) in the mitochondria and is transported to the cytoplasm by the same process as that described for fatty acid synthesis. Acetyl-CoA can also be derived from cytoplasmic oxidation of ethanol. All the reduction reactions of cholesterol biosynthesis use NADPH as a cofactor. Acetyl-CoA units are converted to mevalonate by a series of reactions that begins with the formation of HMG-CoA. Unlike the HMG-CoA formed during ketone body synthesis in the mitochondria, this form is synthesized in the cytoplasm. However, the pathway and the necessary enzymes are the same as those in the mitochondria. Two moles of acetyl-CoA are condensed in a reversal of the *thiolase* reaction, forming acetoacetyl-CoA. Acetoacetyl-CoA and a third mole of acetyl-CoA are converted to HMG-CoA by the action of *HMG-CoA synthase*. HMG-CoA is converted to mevalonate by *HMG-CoA reductase*, HMGR (this enzyme is bound in the endoplasmic reticulum, ER). HMGR absolutely requires NADPH as a cofactor and two moles of NADPH are consumed during the conversion of HMG-CoA to mevalonate. *The reaction catalyzed by HMGR is the rate limiting step of cholesterol biosynthesis, and this enzyme is subject to complex regulatory controls.* You will learn more about this later in the section on regulation of cholesterol synthesis.

Mevalonate is then activated by three successive phosphorylations, yielding 5-pyrophosphomevalonate. In addition to activating mevalonate, the phosphorylations maintain its solubility, since otherwise it is insoluble in water. After phosphorylation, an ATP-dependent decarboxylation yields isopentenyl diophosphate, IDP, an activated isoprenoid molecule. Isopentenyl diophosphate is in equilibrium with its isomer, dimethylallyl diophosphate, DMDP. One molecule of IDP condenses with one molecule

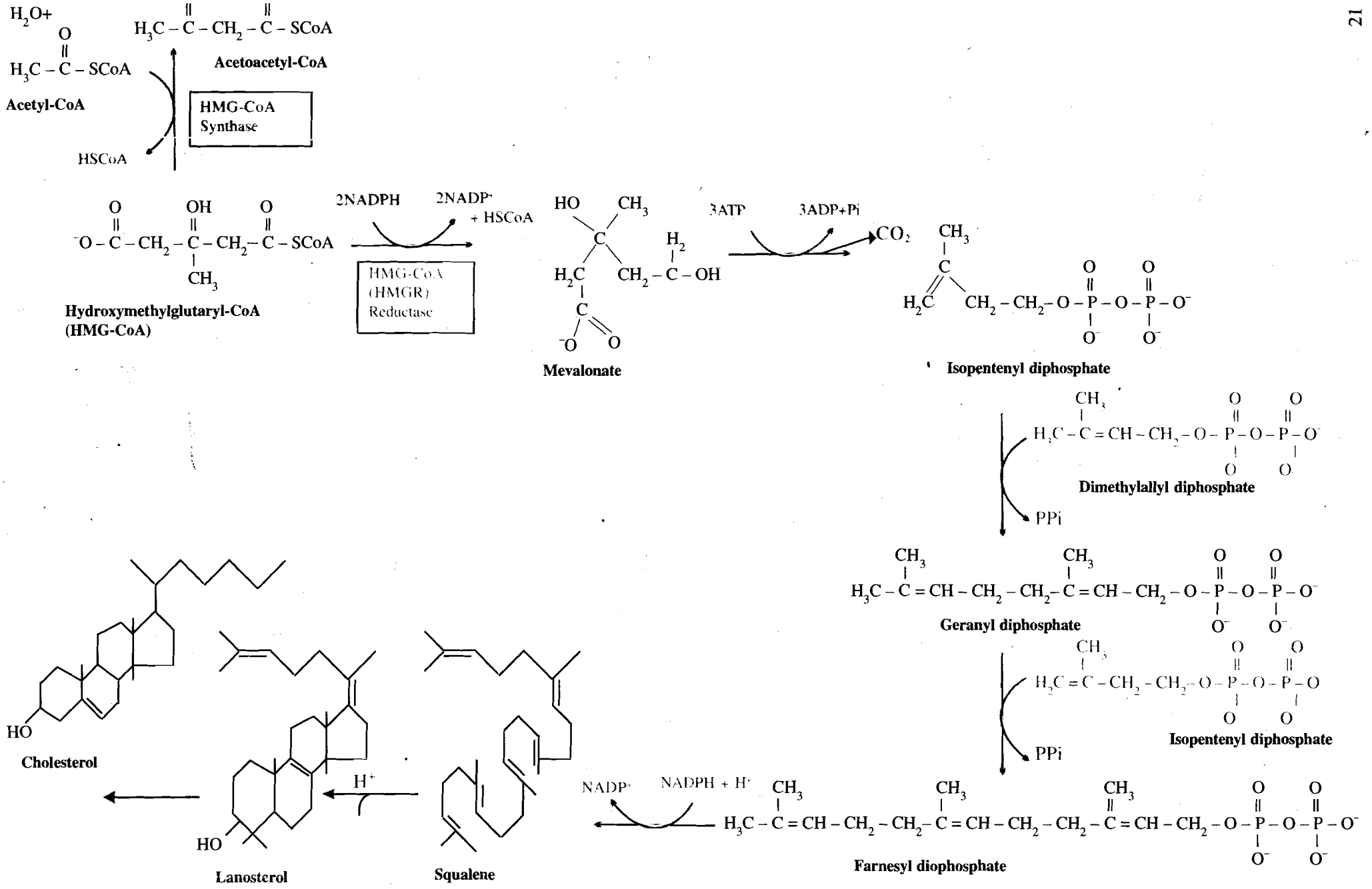


Figure 7.10 : Synthesis of cholesterol

of DMDP to generate geranyl diphosphate, GDP. GDP further condenses (to combine) with another IDP molecule to yield farnesyl diphosphate, FDP. Finally, the NADPH-requiring enzyme, *squalene synthaze* catalyses the head-to-tail condensation of two molecules of FDP, yielding squalene (squalene synthase also is tightly associated with the endoplasmic reticulum). Squalene undergoes a two step cyclisation to yield lanosterol. By the term cyclization we mean, changing an open-chain hydrocarbon to a closed ring. The first reaction is catalyzed by *squalene monooxygenase* or squalene epoxidase. This enzyme uses NADPH as a cofactor to introduce molecular oxygen as an epoxide at the 2,3 position of squalene. Through a series of 19 additional reactions, lanosterol is converted to cholesterol.

B) Regulation of Cholesterol Synthesis

The cellular supply of cholesterol is maintained at a steady level by three distinct mechanisms. Of these three mechanisms, regulation of HMG CoA reductase (HMGR) activity is the primary means for controlling the level of cholesterol biosynthesis.

HMG CoA reductase is an intrinsic membrane protein of the ER. The enzyme's active site extends into the cytosol. HMGR is the rate limiting enzyme in cholesterol synthesis and is subject to different types of metabolic control, which include:

a) *Feedback inhibition*: Cholesterol is a feedback inhibitor of HMG CoA reductase, thus decreasing further cholesterol synthesis.

b) *Hormonal regulation*: HMG CoA reductase activity is controlled hormonally. Glucagon favours the formation of the inactive (phosphorylated) form of HMG CoA reductase and hence decreases the rate of cholesterol synthesis. In contrast, insulin favours formation of the active (unphosphorylated) form of HMG CoA reductase and results in an increase in the rate of cholesterol synthesis.

c) *Sterol-mediated regulation of transcription*: The synthesis of cholesterol is also regulated by the amount of cholesterol taken up by the cells during lipoprotein metabolism. Chylomicron remnants internalized by liver cells, and LDL internalized by cells of liver and peripheral tissues, provide cholesterol, which causes a decrease in de novo cholesterol synthesis. You have already studied about chylomicrons and LDL in Unit 2. Chylomicron and lDL are lipoprotein complexes. You will read in details about these complexes in the next section on lipoprotein synthesis.

d) *Inhibition by drugs*: Lovostatin and mevastatin are reversible competitive inhibitors of HMG CoA reductase. They are used to decrease plasma cholesterol levels in patients with hypercholesterolemia.

Finally, let us look at the degradation of cholesterol in our body.

C) Degradation of Cholesterol

The ring structure of cholesterol cannot be metabolized to CO_2 and H_2O in humans. Rather, the intact sterol ring is eliminated from the body by:

a) conversion to bile acids which are excreted in the faeces,

b) secretion of cholesterol into the bile, which transports it to intestine for elimination is modified by bacteria before excretion.

Figure 7.11 summarizes the sources of liver cholesterol and routes by which cholesterol leaves the liver.

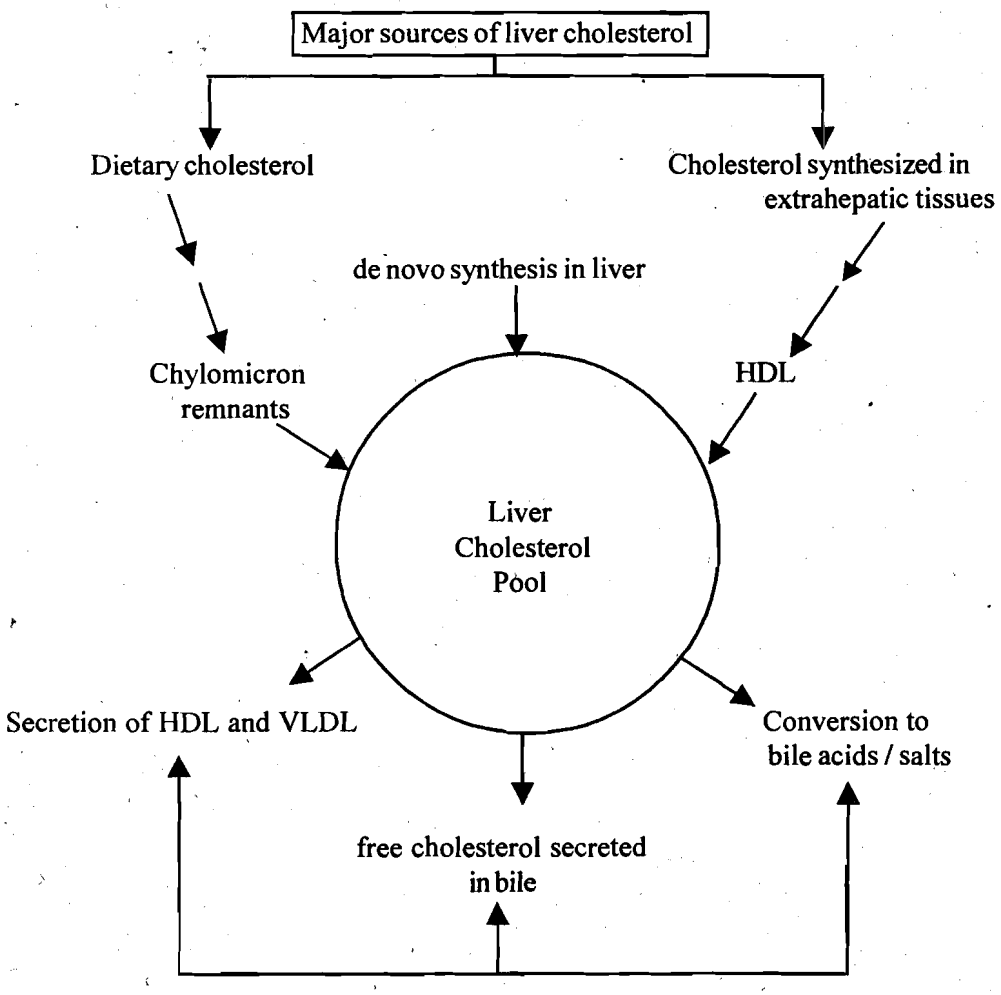


Figure 7.11 : Major routes by which cholesterol leaves liver

Check Your Progress Exercise 3

1) What is the site of synthesis of triacyglycerol? Graphically represent the steps of conversion of fatty acyl CoA to triacyglycerol.

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2) How are phospholipids classified? How are these synthesized? .

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3) Enumerate the five main steps involved in cholesterol biosynthesis.

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4) How is the biosynthesis of cholesterol regulated by the amount of cholesterol in the diet?

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Having studied about cholestrol metabolism, we move on next to the study of lipoprotein metabolism.

7.3.4 Lipoprotein Metabolism

Lipoproteins, as we already know, are the compounds of protein that carry fats and fat-like substances, such as cholesterol, in the blood. You may recall reading earlier about these compounds in Unit 5. The principle lipids carried by lipoprotein particles are triacylglycerols and cholesterol (free or esterified), obtained either from the diet or de novo synthesis. Let us learn a bit more about these plasma proteins.

A) Plasma Lipoproteins – An Introduction

The plasma lipoproteins are molecular complexes of lipids and specific proteins called *apolipoproteins*. In fact, lipoproteins are composed of a neutral lipid core (containing triacylglycerol in cholesteryl esters or both) surrounded by a shell of apolipoproteins (apoproteins), phospholipids and non-esterified cholesterol-all oriented so that their polar portions are exposed on the surface of the lipoprotein. This makes the particle soluble in aqueous medium. *What are apolipoproteins*? You have seen that various types of proteins are present along with different types of lipids in the lipoproteins. These proteins are specifically referred to as *apolipoproteins*. You have already come across this concept earlier while studying about enzymes in Unit 4. Do you recall reading about apoproteins? Yes, we learnt that many enzymes consist of the protein molecule along with the non-protein molecule. The protein molecule is referred to as *apoenzyme*. Apolipoprotein and apoenzymes are also called by a general term *apoprotein*, indicating that a non-protein portion is also associated with it.

These dynamic particles, the lipoproteins – are in constant state of synthesis, degradation and removal from the plasma. Lipoproteins function both to keep lipids soluble as they transport them in the plasma, and to provide an efficient mechanism for delivering their lipid contents to the tissues. In humans, the delivery system is less perfect than in other animals, and as a result, humans experience a gradual deposition of lipids– especially cholesterol in tissues. This is potentially life-threatening occurrence when the lipid deposition contributes to plaque formation, causing the narrowing of blood vessels– a condition known as *atherosclerosis* about which you may recall studying in the course "Applied Physiology" in Unit 4.

Do you recall the lipoproteins highlighted in Unit 5? For your information, Table 7.3 here presents the different lipoproteins and their composition. You would notice that the different lipoproteins are classified based on their size and density.

Table 7.3 : Composition of the plasma lipoprotein (%)

Plasma Lipoproteins	Triacylglycerol	Protein	Phospholipid	Cholesterol
Chylomicrons	90	2	3	5
VLDL	60	5	15	20
LDL	8	20	22	50
HDL	5	25	30	40

The *chylomicrons* are the lipoprotein particles lowest in density and largest in size, and contain the most lipid and the smallest percentage of protein as can be seen in Table 7.3. Chylomicrons function to deliver dietary triacylglycerols to adipose tissue and muscle and dietary cholesterol to the liver.

VLDLs and LDLs are successively denser, having a higher content of protein and a lower content of lipid. HDL particles are the densest of the plasma lipoproteins.

The apolipoproteins associated with lipoprotein particles have a number of diverse functions. It:

a) serves as structural components of the particles,

b) provides recognition sites for cell-surface receptors, and

c) serves as activators or coenzymes for enzymes involved in lipoprotein metabolism.

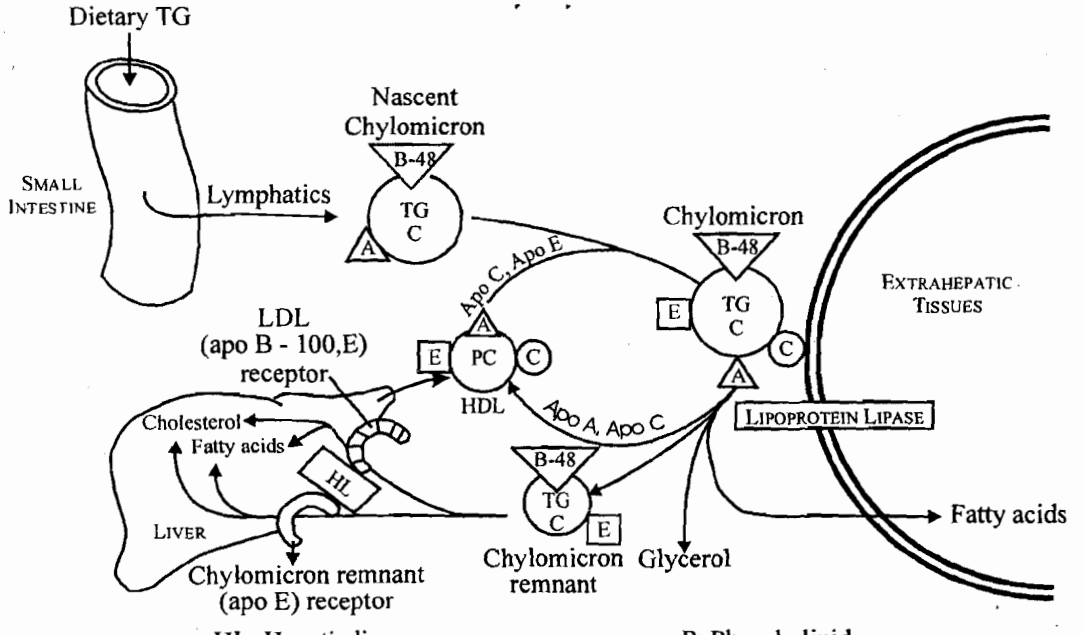
Apolipoproteins are divided by structure and function into classes A to H, with most classes having subclasses, for example, apoA-I and apoC-II.

Having got a basic insight into the structure, composition of lipoproteins, we shall now move on to read about the metabolism of these compounds starting with chylomicrons.

B) Metabolism of Chylomicrons

Chylomicrons are assembled in intestinal mucosal cells and carry dietary triacylglycerol, cholesterol and cholesteryl esters (plus additional lipids made in these cells) to the peripheral tissues.

The particle released by the intestinal mucosal cell is called a *"nascent"* chylomicron and contains apolipoprotein B-48 (apoB-48 as shown in the Figure 7.12). Chylomicrons leave the intestine via the lymphatic system and enter the blood circulation. When it reaches the plasma, the nascent chylomicron is rapidly modified, receiving apoE and apoCII apolipoproteins (from plasma HDLs) which is required for the activation of lipoprotein lipase.



HL: Hepatic lipase
TG: Triglycerides
C: Cholesterol and cholesteryl ester
PC: Phosphatidylcholine
LDL: Low density lipoprotein

P: Phospholipid
A: Apolipoprotein
B-48: Apolipoprotein B-48
Ⓒ: Apolipoprotein C
E: Apolipoprotein E

Figure 7.12 : Metabolism of Chylomicrons

In the capillaries of adipose tissue and muscle, the fatty acids of chylomicrons are removed from the triacylglycerols by the action of *lipoprotein lipase* (LPL), which is found on the surface of the endothelial cells of the capillaries. Lipoprotein lipase is an extracellular enzyme that hydrolyses triacylglycerol into two monoacylglycerol and two fatty acids as indicated in the Figure 7.12. The apoC-II in the chylomicrons activates LPL in the presence of phospholipid. The free fatty acids then enter the cells passively down a concentration gradient and the glycerol backbone of the triacylglycerols is returned via the blood, to the liver and kidneys. Patients with a deficiency of *lipoprotein lipase* or apoC-II show a dramatic accumulation of triacylglycerol-rich lipoproteins in the plasma, for example, *type I hyperlipidemia (familial hyperchylomicronemia).*

As the chylomicron circulates and the triacylglycerol in its core is degraded by *lipoprotein lipase*, the particle decreases in size and increases in density, since it has lost a considerable amount of its lipid component. In addition, the C apoproteins are returned to the HDLs (from which they were originally obtained). The remaining particle left is called a *"remnant"*. Chylomicron remnants-*containing primarily cholesterol, apoE and apoB-48* are then delivered to, and taken up by the liver through interaction with the chylomicron remnant receptor. The recognition of chylomicron remnants by the hepatic remnant receptor requires apoE. Chylomicron remnants bind to these receptors and are taken into the cells by endocytosis. The endocytosed vesicle then fuses with a lysosome, and the apolipoproteins, cholesteryl esters, and other components of the remnant are hydrolytically degraded, releasing amino acids, free cholesterol and fatty acids. The cholesterol released from the chylomicron regulates the rate of de novo cholesterol synthesis in the liver by causing a decrease in cell content of HMG CoA reductase, which you learnt earlier is the key enzyme in cholesterol synthesis, as well as by inhibiting the enzyme.

C) **Metabolism of Very Low Density Lipoproteins (VLDL)**

VLDLs are produced in the liver. They are composed predominantly of triacylglycerols (TG), cholesterol and cholesteryl esters (C) and their function is to carry this lipid from the liver to the peripheral tissues. There, the triacylglycerol is degraded by *lipoprotein lipase*, as discussed, for chylomicron degradation. The process involved, thereafter is illustrated in Figure 7.13 and the process includes:

1) *Release of VLDL:* VLDLs are released from the liver as nascent VLDL particles containing apolipoproteins B-100 and A-I. They must obtain apoC-II and apoE from circulating HDL as shown in Figure 7.13. As with chylomicrons, apoC-II is required for activation of *lipoprotein lipase.*

2) *Modification of circulating VLDL:* Next, as VLDLs pass through the circulation, their structure is altered. Fatty acids and glycerol are removed by *lipoprotein lipase*, causing the VLDL to decrease in size and become denser to form intermediate density lipoproteins (IDL). Surface components, including the C and E apolipoproteins are transferred to HDL. Finally, cholesteryl esters are transferred from HDL to VLDL in an exchange reaction that concomitantly transfers triacylglycerol or phospholipid from VLDL to the HDL. This exchange is accomplished by *cholesteryl ester transfer protein.*

3) *Production of LDL from VLDL in plasma:* After these modifications, the VLDL has been converted in the plasma to LDL. An intermediate-sized particle, the *intermediate density lipoprotein (IDL)* as shown in Figure 7.13, is observed during the transition from VLDL to LDL in the plasma. IDLs can also be taken up by cells through receptor-mediated endocytosis.

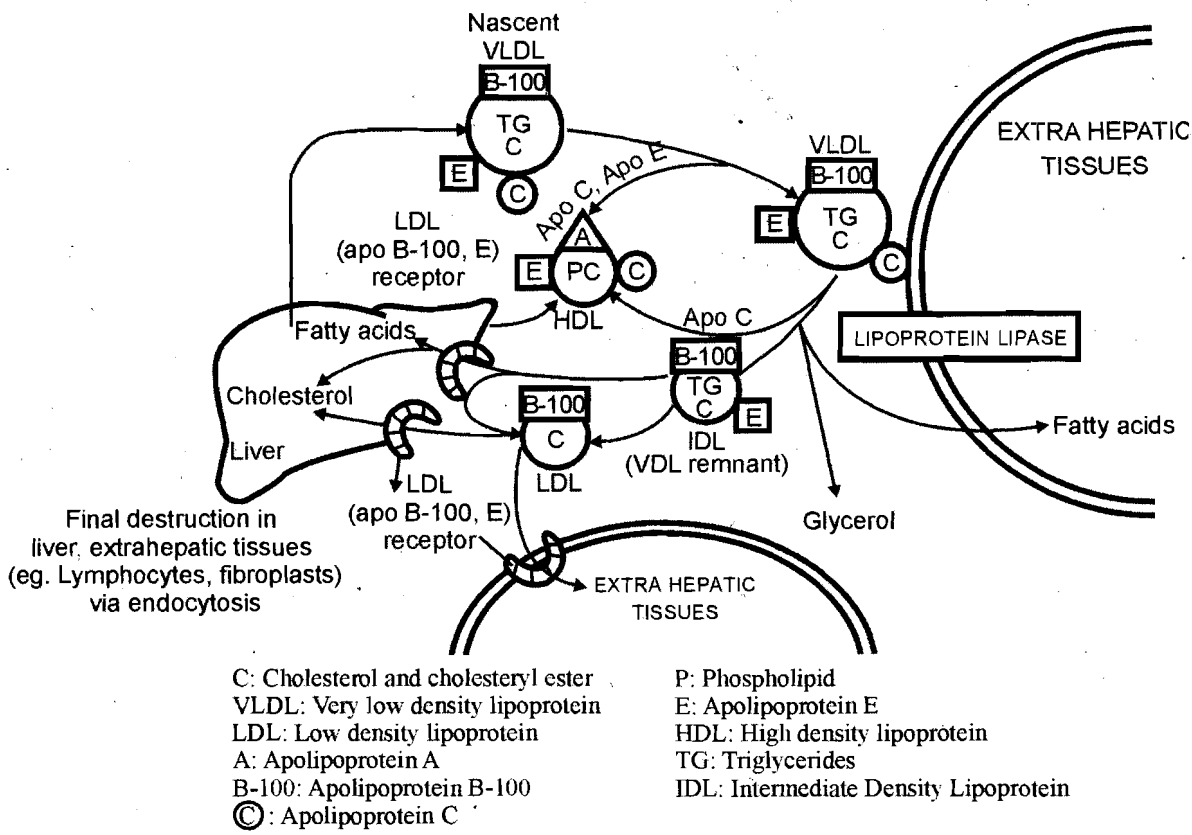


Figure 7.13: Metabolism of VLDL

Next, let us look at the metabolism of LDL.

D) Metabolism of Low Density Lipoproteins (LDL)

LDL, as seen earlier, contains much less triacylglycerol than its VLDL predecessors, and has a high concentration of cholesterol and cholesteryl esters. The primary function of LDL particles is to provide cholesterol to the peripheral tissues. How do they do that? In fact, a multistep process is involved in the metabolism of LDL. We shall not go into the details of these steps, but certainly look at the mechanism involved in simple terms.

LDL particles provide cholesterol to the peripheral tissues by depositing free cholesterol on the membranes of cells as they come in contact with the cell surface and by binding to receptors on cell-surface membranes that recognize apolipoprotein B-100. LDL receptors are negatively charged glycoprotein molecules that are clustered in pits on cell membranes. The intracellular side of the pit is coated with the protein *clathrin*, which stabilizes the shape of the pit. After binding, the LDL is internalized as intact particles by *endocytosis*. The vesicle containing the LDL rapidly loses its clathrin coat and fuses with other similar vesicles, forming larger vesicles called *endosomes*. The pH of the contents of the endosome falls allowing separation of the LDL from its receptor. The receptors then migrate to one side of the endosome, whereas the LDLs stay free within the lumen of the vesicle.

The receptors can be recycled, whereas, the lipoprotein remnants in the vesicle are degraded by lysosomal (hydrolytic) enzymes, releasing cholesterol, amino acids, fatty acids and phospholipids. These compounds can be recycled by the cell. The number of receptors for lipoproteins vary according to the availability of these lipoprotein particles and according to the needs of the cell. For example, if there is a large amount of a particular circulating plasma lipoprotein, the number of cell-surface receptors for it decreases, frequently termed "down-regulation". Conversely, if cells are starved for cholesterol, they increase the number of cell-surface receptors, i.e. "up-regulation".

Lastly, we come to the metabolism of HDL.

E) Metabolism of High Density Lipoproteins (HDL)

HDL particles are synthesized in the liver and are released into the bloodstream by exocytosis. HDL performs a number of important functions as you may have realized while reading through the earlier sections. These include:

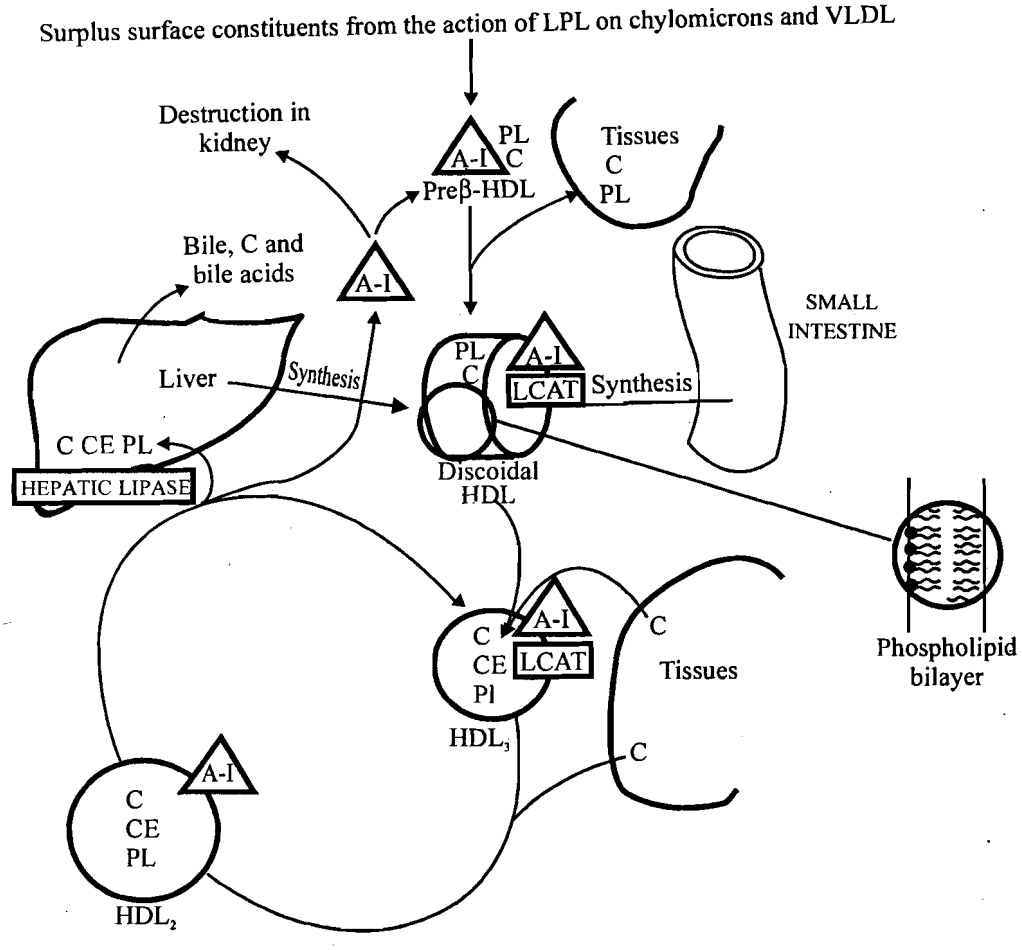
a) HDL serves as a circulating reservoir of apolipoprotein - apoC-II (the apolipoprotein that is transferred to VLDL and chylomicrons).

b) It is an activator of *lipoprotein lipase*, removing free (unesterified) cholesterol from extrahepatic tissues and esterifying it, using the plasma enzyme phosphatidylcholine cholesterol acyltransferase (PCAT – also known as LCAT, where "L" stands for lecithin).

c) It transfers cholesteryl esters to VLDL and LDL in exchange for triacylglycerol, and

d) It carries cholesteryl esters to the liver, where the HDL is degraded and cholesterol is released.

A brief discussion of the functions and metabolism of HDL follows. The metabolism of HDL is illustrated in Figure 7.14.



HDL: High density lipoprotein
L CAT: Lecithin:cholesterol acyltransferase
LPL: Lipoprotein Lipase
C: Cholesterol
CE: Cholesteryl ester
PL: Phospholipid
A-I: Apolipoprotein A-I

Figure 7.14 : Metabolism of HDL

1) *HDL as a reservoir of apolipoproteins:* HDL particles not only serve as the source of apolipoproteins required for the proper metabolism of other plasma lipoproteins, but also take back most of these proteins before the chylomicron remnants and LDLs bind to their cell-surface receptors and are endocytosed.

2) *HDL uptake of free cholesterol:* Newly secreted HDL are disc-shaped particles as shown in the Figure 7.14, containing predominantly unesterified cholesterol, phospholipids and a number of apolipoproteins including apoE, apoA and apoC. They are rapidly converted to spherical particles as they accumulate cholesterol. HDL particles are excellent acceptors of unesterified cholesterol from the surface of cell membranes and from other circulating lipoproteins.

3) *Esterification of free cholesterol:* Once free cholesterol is taken up by the HDL, it is immediately esterified by *phosphatidyl cholesterol acyl transferase* (PCAT), a plasma enzyme synthesized by the liver, which is activated by apoA-I of the HDL. Plasma levels of apoA-I are increased by modest alcohol intake. The fatty acid from carbon 2 of phosphatidylcholine is transferred directly to the cholesterol, leaving lysophosphatidylcholine. The resulting cholesteryl ester is so hydrophobic that it is effectively "trapped" in the HDL and can no longer be transferred to a membrane. The only mechanism for removing it from HDL in the plasma is through transfer to VLDL by the cholesteryl ester transfer protein, where it ultimately remains in the LDL until that particle is taken up by a cell. About two-thirds of the cholesterol in the plasma is esterified with fatty acid. In liver disease, a decreased concentration of plasma cholesteryl esters is observed. This may be due to either a deficiency in phosphatidylcholine production or a lack of PCAT.

4) *Fate of HDLs:* Spherical HDL particles are taken up by the liver by receptor-mediated endocytosis and the cholesteryl esters are degraded. The cholesterol thus released by the action of enzyme hepatic lipase can be repackaged in lipoproteins, converted into bile acids, or secreted into the bile for removal from the body.

With the metabolism of HDL, we come to the end of our discussion on metabolism of lipoproteins. We have seen in this section, the fate of the different lipoproteins. Serum lipoprotein levels are maintained in the body. What happens when the levels of these lipoproteins are elevated in the body? Read the next section and find out.

Check Your Progress Exercise 4

1) Define the following terms:

   a) Lipoproteins

   .....

   .....

   b) Apolipoproteins

   .....

   .....

2) Name the lipoprotein particles that have the highest percentage concentration of:

   a) Cholesterol .....

   b) Triacylglycerol .....

   c) Protein .....

   d) Phospholipids .....

3) What is the reaction catalyzed by lipoprotein lipase? Which lipoproteins will get elevated in case of decreased activity of lipoprotein lipase? Which is the compound that gets accumulated in the plasma? What is the condition referred to as?

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4) Give the origin and fate of LDL cholesterol.

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5) What is the shape and content of:

a) Immature HDL

.....

b) Mature HDL

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6) Explain how free cholesterol is esterified.

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7.4 HYPERLIPOPROTEINEMIAS

The term *hyperlipoproteinemia* describes a group of disorders in which serum lipoprotein levels are elevated. These disorders are classified into six types, depending on which lipoproteins are abnormally elevated and are summarized in Table 7.4. Each hyperlipoproteinemia is not a single disease but a group of disorders marked by the same lipoprotein abnormality, and each includes some primary (genetically transmitted) disorders and some secondary disorders. When a disorder of lipid metabolism occurs secondary to a particular disease (e.g. type IV hyperlipoproteinemia secondary to uncontrolled diabetes), treatment of the underlying illness will frequently correct the lipid abnormality. Similarly, when a primary lipid disorder is aggravated by exogenous obesity, alcohol or glucocorticoids, the elimination of the aggravating factor will make diet therapy easier. Diet also affects the development of hyperlipoproteinemias. The dietary factors causing an increase in plasma lipoproteins in a great many people are obesity and high intake of foods rich in cholesterol and saturated fats.

Table 7.4 : Types of hyperlipoproteinemia

Type	Triglyceride	Total Cholesterol	LDL Cholesterol	Raised Lipoprotein
I	+++	+	N	Chylomicrons
II a	N	++	++	LDL
II b	++	++	++	LDL/VLDL
III	++	+	N	IDL and chylomicron remnants
IV	++	N/+	N	VLDL
V	++	+	N	VLDL / chylomicrons

N= Normal; + = slightly raised; ++ = moderately raised; +++ = extremely raised

A brief review follows:

a) **Type I** hyperlipoproteinemia is an uncommon pattern marked by elevated chylomicrons. Cholesterol is normal and triglycerides are markedly elevated, usually greater than 1000 mg/dl. Among the primary disorders producing this pattern are familial lipoprotein lipase deficiency and apolipoprotein C II deficiency.

b) **Type II a** hyperlipoproteinemia is marked by high LDL with normal VLDL. Plasma cholesterol levels are high but triglycerides are normal. The genetic disorder associated with this pattern is *familial hypercholesterolemia,* in which there is an autosomal dominant pattern of inheritance. The biochemical defect is a deficiency of LDL receptors. This pattern is also seen secondary to nephrotic syndrome, Cushing's syndrome and hypothyroidism.

c) **Type II b** hyperlipoproteinemia is a common pattern characterized by increases in VLDL and LDL. Both cholesterol and triglyceride levels are elevated. *Familial combined hyperlipoproteinemia,* also called familial multiple lipoprotein-type hyperlipidemia, is a disorder in which individuals with type II a, type II b and type IV hyperlipoproteinemias are found in the same family. Type IIb hyperlipoproteinemia can be seen *secondary* to nephrotic syndrome, Cushing's disease and hypothyroidism. *Primary* type II b hyperlipoproteinemia can be aggravated by exogenous obesity or glucocorticoids.

d) **Type III** hyperlipoproteinemia is marked by a reduced electrophoretic mobility of VLDL. In this, cholesterol and triglycerides are both elevated, frequently to about the same level– for example, cholesterol and triglycerides may both be 400 mg/dl.

The primary form of this disorder is called *familial dysbetalipoproteinemia.* These patients accumulate a partially degraded VLDL (beta VLDL).

e) **Type IV,** a common pattern of hyperlipoproteinemia, is marked by elevated VLDL with high cholesterol and triglycerides.

1) The *primary disorders* associated with this pattern are familial multiple lipoprotein-type hyperlipidemia, and the mild form of familial hypertriglyceridemia.

2) In other associated disorders, elevated VLDL is common secondary to diabetes and uremia, and is also associated with hypopituitarism and nephrotic syndrome. Alcohol, glucocorticoids, oestrogens and exogenous obesity may aggravate an already elevated VLDL in patients with primary hyperlipidemia but they seldom induce hyperlipidemia in normal individuals.

f) **Type V,** a rare pattern, is marked by elevated chylomicrons and VLDL. Both cholesterol and triglycerides are high.

1) The *primary disorders* with the pattern are familial lipoprotein lipase deficiency, apolipoprotein C II deficiency, and the more severe form of the familial hypertriglyceridemias.

2) Type V hyperlipoproteinemia may be seen *secondary to the same disorders as type IV*, it is most commonly seen secondary to poorly controlled diabetes.

Our reading on this topic would not be complete without some information about the diagnosis of these disorders. We shall learn about this next.

Diagnosis of Hyperlipoproteinemia

We have already seen how hyperlipoproteinemia is classified based on the elevated levels of lipoproteins such as chylomicron, LDL or VLDL etc. Next, we shall learn why the diagnosis of hyperlipoproteinemia is important and when to do it. Some information of the diagnosis mechanism is also included.

1) Because of the high association of hyperlipidemia with coronary heart disease, it is generally recommended that serum cholesterol and triglycerides be measured periodically, especially in young adults. If there is a history of hyperlipoproteinemia or premature coronary artery disease in the family, children should be tested as well.

   a) *Timing of measurement:* The serum cholesterol level is relatively unaffected by eating, but a recent meal can cause marked elevation of triglycerides. Triglycerides should only be measured after a 12 to 14-hour fast. Serum lipids are best determined when the patient is maintaining a steady weight and has been on his usual diet for several weeks.

   b) *Repeat measurements:* Before making a firm diagnosis, fasting lipids should be measured two or three times at 2 to 3-week intervals.

2) The presence of chylomicrons can be determined by refrigerating the plasma at 4°C overnight. If chylomicrons are present, they will form a creamy layer on top of the plasma. The presence of chylomicrons in plasma drawn after a 12-hour fast is indicative of *type I or type V hyperlipoproteinemia.* Fasting chylomicronemia is usually seen only with fasting triglyceride levels of greater than 1000 mg/dl.

3) The implications of elevated serum cholesterol depend on the lipoprotein, with which it is associated. As noted above, the risk of coronary artery disease is highly associated with elevated LDL cholesterol. A marked increase in VLDL may result in some increase in cholesterol in addition to an increase in triglyceride. VLDL contains about 1 mg of cholesterol for every 4 mg of triglyceride. HDL cholesterol is easily determined by precipitating LDL and VLDL with phosphotungstic acid and magnesium chloride. The nonprecipitated cholesterol is HDL. LDL cholesterol can then be calculated :

$$\text{LDL cholesterol} = \text{Total cholesterol} - \text{HDL cholesterol} - \frac{\text{triglycerides}}{5}$$

4) Most patients with hyperlipidemia can be *assigned to a specific hyperlipoproteinemia* on the basis of total cholesterol, triglycerides, HDL cholesterol and the refrigerator test for chylomicrons. Isoelectric focusing of apolipoproteins is necessary to establish the diagnosis of CII apolipoprotein deficiency and familial dysbetalipoproteinemia (type III hyperlipoproteinemia).

Finally, we shall end our discussion on lipid metabolism by learning about *ketosis*. What is ketosis? Ketosis is the body's survival system. Let's get to know about this system in our body.

7.5 KETOSIS

Being in ketosis means our body has burned a large amount of fat in response to the fact that it did not have sufficient glucose available for energy needs. Under everyday conditions, the carbohydrates we eat are converted to glucose, which you already know is the body's primary source of energy. Whenever our intake of carbohydrates is limited, for a long enough period of time, we will reach a point where our body draws on its alternate energy system i.e. the fat stores for fuel.

The condition called ketosis, means our body burns fat and turns it into a source of fuel called *ketone bodies*. Ketone bodies are produced whenever body fat is burned. When you burn a larger amount of fat that is immediately needed for energy, the excess ketone bodies are discarded in the urine. Let us next see how ketone bodies are formed?

Acetyl CoA is oxidized to CO_2 via citric acid cycle, as given in carbohydrate metabolism. Only in liver mitochondria, the acetyl CoA can be converted to ketone bodies, i.e. *acetoacetate, acetone* and *3-hydroxybutyrate*. The ketone bodies are *water soluble lipid fuels that are continuously released from the liver.* When carbohydrate is plentiful and glucose is readily available to the tissues, the amount of circulating ketone bodies is low. When large amounts of triacylglycerols are being hydrolyzed in adipose tissue, in response to an increase in whole body energy demand, the rate of oxidation of fatty acid increases in the liver and other tissues. In the liver these increases ketogenesis and thus increases the ketone body concentration in the circulation. Normally, some acetoacetate is converted to 3-hydroxybutyrate. Further, acetoacetate and hydroxybutyrate are valuable fuels for skeletal and cardiac muscle. It is estimated that they supply 10 per cent of the daily energy requirement of these tissues. The breakdown of ketone bodies by the peripheral tissues is called *ketolysis*. When the process of ketogenesis exceeds ketolysis, ketosis or ketoacidosis occurs. Here we must differentiate *ketosis* from *ketoacidosis*. Ketosis we have seen is our body's natural survival system. *Ketoacidosis*, on the other hand, is *a life-threatening condition most often associated with uncontrolled insulin-deficient Type 1 diabetes*. In Type 1 diabetes, the absence of insulin leads to a toxic build-up of blood glucose and an extreme breakdown of fat and muscle tissue. The presence of insulin keeps ketone bodies production in check so that a mild, beneficial ketosis is achieved.

Check Your Progress Exercise 5

1) What do you mean by the term 'hyperlipoproteinemia'? How are these classified?

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2) How can you determine the presence of chylomicrons, HDL and LDL in the plasma?

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3) What is ketosis? Is it same as ketoacidosis?

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4) What are ketone bodies? How are these produced? Name three ketone bodies.

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5) Match the following:

A		B	
i)	Type I hyperliproproteinemia	a)	High cholesterol and triglycerides
ii)	Type II a hyperlipoproteinemia	b)	Elevated chylomicrons and triglycerides
iii)	Type III hyperlipoproeinemia	c)	High LDL and plasma cholesterol
iv)	Type IV hyperlipoproteiemia	d)	High VLDL, cholesterol and triglycerides

7.6 LET US SUM UP

In this unit, lipid metabolism at cellular level has been discussed at length. First we have seen how fatty acids are used for the production of energy. Secondly, the body has the capacity to synthesize a variety of fatty acids except the essential fatty acid. Higher fatty acids i.e. eicasonoids have important biological functions. The significance of cholesterol in biologic processes along with their synthesis and regulation has been looked into. Lastly, we have seen the transport of lipoproteins and its relation to hyperlipoproteinemia. Six types of hyperlipoproteinemia are present. Ketosis occurs when the process of ketogenesis exceeds the process of ketolysis.

7.7 GLOSSARY

Apolipoprotein : the protein component of lipoproteins.

Atherosclerosis : a thickening and narrowing of the walls of the large and medium sized blood vessels caused by the invasion of lipids, primarily cholesterol and other materials, into the internal or inner layer to form plaque.

Condensation : a chemical change in which two molecules combine to form a larger molecule with elimination of a small molecule e.g. H_2O.

Endocytosis : process of cellular ingestion by which the plasma membrane folds inward to bring substances into the cell.

Endoplasmic Reticulum : a membrane network within the cytoplasm of cells involved in the synthesis, modification and transport of cellular materials.

Esterification : the process of converting an acid into an alkyl or aryl derivative and consists of the reaction of an acid with an alcohol in the presence of a trace of mineral acid as catalyst or the reaction of an acyl chloride with an alcohol. It can also be accomplished by enzymatic processes.

Exocytosis : a process of cellular secretion or excretion in which substances contained in vesicles are discharged from the cell by fusion of the vesicular membrane with the outer cell membrane.

Head-to-tail condensation : a chemical change in which two molecules combine head-to tail to form a larger molecule with elimination of a small molecule e.g. H_2O is called head-to-tail condensation.

Infarction : an area of coagulation necrosis in a tissue due to local ischemia resulting from obstruction of circulation to the area.

Ketoacidosis : a life-threatening condition most often associated with uncontrolled IDDM.

Ketone bodies : water-soluble lipid fuels that are continuously released from the liver.

Ketosis : burning or utilization of a large amount of fat in response to decreased glucose availability for energy needs.

Leukotrienes : compounds derived from arachidonic acid and are linear oxidation products found in leukocytes; contain a conjugated triene double bond arrangement.

Prostaglandins : C-20 unsaturated hydroxy acids with a substituted cyclopentane ring and two aliphatic side chains. It is one of the extremely potent compounds that elicit a wide range of physiologic responses.

Thromboxanes : compounds that cause the aggregation of platelets that is involved in the formation of a blood clot. Thromboxanes have an oxygen atom incorporated into a cyclopentane ring which produces a six membered ring.

7.8 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1) Their oxidation consists of : (a) activation, (b) transport into the mitochondrial matrix, and (c) reactions of β-oxidation. Activation is catalyzed by *fatty acyl-CoA synthetase*. At least three acyl-CoA synthetases, each specific for a particular size (length) of fatty acid are known.

   - Acetyl CoA synthetase: acts on acetate and low molecular weight fatty acids,
   - Medium chain acyl CoA synthetase: acts on fatty acids with 4-11 carbon atoms, and
   - Acyl CoA synthetase: acts on fatty acids with 6 to 20 carbon atoms.

2) The enzymes involved include: Carnitine Palmitoyl Transferase I and Carnitine Palmitoyl Transferase II. The mitochondrial wall is impermeable to fatty acyl CoA. However, fatty acyl CoA reacts with carnitine in the presence of CPT1 and CoA is released and fatty acid forms complex with carnitine in the presence of CPT1 and CoA is released and fatty acid forms complex with carnitine called acyl-carnitine which is able to penetrate the mitochondrial wall.

3) The major pathway for fatty acid oxidation is β-*oxidation.* The process of fatty acid oxidation is termed as β-oxidation since it occurs through the sequential removal of 2-carbon units (as acyl CoA) by oxidation at the β-carbon position (between α(2) and β(3) carbons from carboxyl carbon) of the fatty acyl-CoA molecule.

4) The stearic acid is converted to stearyl CoA. Then 8 rounds of beta oxidation yield 9 molecules of acetyl CoA which enter the citric acid cycle. There are 3 parts: 1) 2 ATPs used to convert stearic acid to stearyl CoA 2). Each round of beta oxidation produces 1 NADH and 1 $FADH_2$ for 5 ATPs. So for 8 rounds: 40 ATP. 3) Each acetyl CoA in Krebs cycle gives 3 NADH and 1 GTP for total 12 ATP. So 9 acetyl CoAs yield 1 108 ATPs.

Total = 45 + 108 – 2 = 146.

5) The oxidation of unsaturated fatty acids is essentially the same process as for saturated fats, except when a double bond is encountered. In such a case, the bond is isomerized by a specific *enoyl-CoA isomerase* and oxidation continues. In the case of linoleate (linoleic acid), the presence of the C-12 unsaturation results in the formation of a dienoyl-CoA during oxidation. This molecule is the substrate for an additional oxidizing enzyme, the NADPH requiring *2,4-dienoyl-CoA reductase.*

Check Your Progress Exercise 2

1)a) Lipogenesis is the process which involves the synthesis of fatty acids from acetyl CoA and the esterification of fatty acids in the production of triacylglycerol.

b) Acetyl CoA Carboxylase is the key regulating enzyme of lipogenesis.

c) The process of conversion of malonyl CoA to β-hydroxybutyryl-ACP involves :
malonyl CoA + ACP-SH → malonyl ACP + CoASH

malonyl ACP + acetyl-cys-CE → beta-ketobutyryl-ACP + CO_2

α - ketobutyryl - ACP + NADPH + H^+ → beta-hydroxybutyryl-ACP + NAD^+

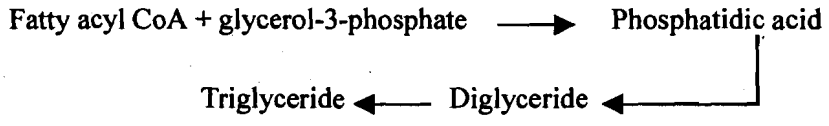
2) Fatty acid synthesis is simply not a reversal of fatty acid degradation, but does start with acetyl CoA and does build up by the addition of two carbons units. The fatty acid synthesis occurs in the cytoplasm in contrast to the degradation (oxidation), which occurs in the mitochondria. The major lipogenic tissues are the intestine, liver and adipose tissue.

3) Palmitic acid may be converted to stearic acid (18: O) by elongation of the carbon chain. Desaturation of stearic acid produces oleic acid (C18 : 1 Δ 9).

4) Prostaglandins and the related compounds such as thromboxanes and leukotrienes are collectively known as eicosanoids. These are derived from Essential Fatty Acids. The dietary precursor of the prostaglandins is the essential fatty acid linoleic acid. It is converted to its immediate precursor of the prostaglandins–20 C, PUFA containing 3, 4 or 5 double bonds. Arachidonic acid is the precursor of the predominant classes of prostaglandins.

Check Your Progress Exercise 3

1) Endoplasmic Reticulum is the site for synthesis of triacylglycerol.



2) Phospholipids are synthesized by esterification of an alcohol to the phosphate of phosphatidic acid (1,2-diacylglycerol 3-phosphate). Phospholipids are classified as : Phosphatidylserine (PS), Phosphatidylglycerol (PG), Phosphatidylethanolamine (PE) and Phosphatidylinositol (PI).

3) • Acetyl-CoAs are converted to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA)
   • HMG-CoA is converted to mevalonate
   • Mevalonate is converted to the isoprene based molecule, isopentenyl diphosphate (IDP), with the concomitant loss of CO_2
   • IDP is converted to squalene
   • Squalene is converted to cholesterol.

4) A feedback mechanism exists in which intracellular cholesterol inhibits HMG CoA reductase. When the diet is rich in cholesterol, intracellular cholesterol increases in the liver and the synthesis of cholesterol is suppressed. On the other hand, a low cholesterol diet but with adequate triglyceride will stimulate cholesterol synthesis.

Check Your Progress Exercise 4

1) a) The compounds of protein that carry fats and fat like substances, such as cholesterol in the blood. Triglycerides, phospholipids, protein, cholesterol and cholesterol esters.

   b) Various types of proteins are present along with different types of lipids in the lipoproteins. These proteins are specifically referred to as apolipoproteins.

2) a) LDL
   b) Triacyglycerol
   c) HDL
   d) HDL

3) Lipoprotein lipase is an extracellular enzyme that hydrolyses triacylglycerol into 2 monoacylglycerol and two fatty acids. The fatty acids then enter the cell passively down a concentration gradient.

   If the activity of lipoprotein lipase is decreased both plasma chylomicrons and VLDL– the two particles that carry predominantly triacylglycerol–would become elevated. LDL and HDL containing little triacylglycerol and would therefore not become elevated in the plasma. Triacylglycerol-rich lipoproteins will get accumulated in the plasma. The condition is called type I hyperlipidemia.

4) LDL are glycoprotein molecules found in cluster in pits on cell membranes. They provide cholesterol to the peripheral tissues. After binding, the LDL is internalized as intact particles by *endocytosis*. The vesicle containing the LDL fuses with other similar vesicles, forming larger vesicles called *endosomes*. The pH of the contents of the endosome falls allowing separation of the LDL from its receptor. The lipoprotein remnants in the vesicle are degraded by lysosomal (hydrolytic) enzymes, releasing cholesterol, amino acids, fatty acids, and phopholipids. These compounds can be recycled by the cell.

5) a) Newly secreted HDL are disc-shaped particles containing predominantly unesterified cholesterol, phospholipid and a number of apolipoproteins including apoE, apoA and apoC.

   b) Spherical, contains cholesterol, cholesteryl esters and phospholipids

6) Once free cholesterol is taken up by the HDL, it is immediately esterified by phosphatidyl cholesterol acyl transferase (PCAT), a plasma enzyme synthesized by the liver, which is activated by apoA-I of the HDL.

Check Your Progress Exercise 5

1) The term 'hyperlipoproteinemia' describes a group of disorders in which serum lipoprotein levels are elevated. These disorders are classified into six types, depending on which lipoproteins are abnormally elevated; Type I, IIa, II b, III, IV and V.

2)
- Chylomicrons – by refrigerating the plasma at 4°C overnight. If chylomicrons are present, they will form creamy layer on top of the plasma.
- HDL–by precipitating LDL and VLDL with Phosphotungstic acid and Magnesium chloride.
- LDL= Total Cholesterol – HDL – $\frac{\text{Triglycerides}}{5}$

3) When our body burns / utilizes a large amount of fat in response to the fact that it did not have sufficient glucose available for energy needs. This condition is termed as Ketosis. No, it is not same as Ketoacidosis, which is most often associated with uncontrolled diabetes.

4) Ketone bodies are water-soluble lipid fuels that are continuously released from the liver. These are produced whenever body fat is burned. The 3 types of ketone bodies are acetoacetate, acetone and 3 hydroxy butyrate.

5) i) - (b)

ii) - (c)

iii) - (a)

iv) - (d)